The phylogeny of *Staphylococcus aureus* clonal complex 398 and its interaction with various hosts

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ZUSAMMENFASSUNG

Staphylococcus aureus ist nicht nur ein häufiger Besiedler des *Vestibulum nasi* aller Säugetiere, sondern auch Verursacher vielfältiger Infektionen in Krankenhäusern und der nicht hospitalisierten Bevölkerung. Beim Menschen reicht das weite Infektionsspektrum von Infektionen der Haut bis hin zur lebensbedrohlichen Endokarditis. Besonders besorgniserregend sind, auf Grund der begrenzten Möglichkeiten der antibiotischen Therapie, Infektionen mit Methicillin-resistenten *S. aureus* (MRSA). Seit 2004 wurden MRSA des klonalen Komplexes CC398 weltweit als Besiedler und Infektionserreger bei Nutz- und Haustieren nachgewiesen. Seit dieser Zeit wächst die Besorgnis um damit verbundene gesundheitliche Risiken für den Menschen.

In der vorliegenden Arbeit wurde die Populationsstruktur des klonalen Komplexes CC398 durch Analyse von Mutationen in 97 chromosomal lokalisierten Haushaltsgenen bei 195 Isolaten untersucht. Diese stammen von 11 verschiedenen Wirtsspezies aus mehreren Ländern. Des Weiteren wurden bei ausgewählten Isolaten einer bestimmten Subpopulation die Fähigkeit an Fibronectin zu binden und die Phagozytoserate durch Granulozyten von verschiedenen Wirtsspezies untersucht. Um tiefere Einblicke in die genetische Ausstattung dieser Subpopulation zu erhalten, wurden Ganzgenomsequenzierungen für repräsentative Isolate durchgeführt.

Die vorliegende Arbeit erweitert unsere Kenntnisse zur Populationsstruktur des klonalen Komplexes CC398. Insbesondere zeigte sich, dass es eine speziell in Pferde-Kliniken verbreitete Subpopulation gibt, die als Besiedler und Infektionserreger bei Pferden und auch als nasaler Besiedler bei Menschen mit engem Kontakt zu diesen Tieren auftritt. Bisher wurden Isolate dieser Subpopulation nur sehr selten als Infektionserreger beim Menschen nachgewiesen. Offenbar kann diese Subpopulation Menschen und Pferde effizient besiedeln, ist aber nicht gegen die Wirtsimmunität geschützt. Die vergleichende Genomanalyse zeigte das Auftreten einer neuen Pathogenitätsinsel, sowie zweier bisher noch nicht beschriebener Prophagen bei Isolaten dieser Subpopulation.

SUMMARY

Staphylococcus aureus is not only a frequent nasal coloniser of all mammals and birds, but also is a common cause of a wide range of infections in both hospitals and the community. In humans, *S. aureus* is associated with a wide spectrum of diseases from skin infections to life-threatening endocarditis. Infections caused by methicillin-resistant *S. aureus* (MRSA) are of particular concern due to limited treatment opportunities. Since the early 2000s, a particular MRSA clonal complex (CC398) was widely disseminated as a coloniser and pathogen in economically important livestock and companion animals worldwide. Since then, CC398 is of increasing concern to pose a risk to public health.

In this study, we investigated the population structure of CC398 through mutation discovery at 97 genetic housekeeping loci, which are distributed along the *S. aureus* chromosome within 195 CC398 isolates, collected from 11 different host species and various countries. Furthermore, we investigated the phenotypic characters of a certain sub-clone within CC398 by studying its ability to adhere to fibronectin and to invade neutrophils from various host species. To gain a better insight into the genetic determinants of this CC398 sub-clone, we have applied whole genome sequencing approach on eight representative CC398 isolates.

This study provided a novel insight into the phylogeny of CC398 concerning the spread of a specific MRSA-CC398 sub-clone within equine settings, which causes infections in horses and nasal colonisation of humans that are in close contact with these horses. Of note, it remained extremely rare among *S. aureus* isolates from human infections. Furthermore, this MRSA-CC398 sub-clone can initiate colonisation in both human and horse efficiently; however, it was not generally protected from the host immune system response. Lastly, the comparative genomic analysis of CC398 revealed a novel pathogenicity island and two prophages that were harboured by certain CC398 isolates.

1 INTRODUCTION

1.1 Staphylococci

In earlier times, several scientists had observed cocci shaped bacteria in clinical specimens such as pus and wound secretions. Although cocci bacteria exhibited different morphological characters under the microscope, the German surgeon Billroth believed that all cocci represented different life cycle stages of the same microorganism. It was the merit of the British surgeon Alexander Ogston to introduce the discrimination between streptococci and staphylococci in 1881. As staphylococci appear as grape-like clusters under the microscope, he deduced the name from *Staphylococcus*, Greek for "bunch of grapes" [1]. Staphylococci belong to the family *Staphylococcaceae* and are Gram-positive cocci, non-motile, non-spore forming, and facultative anaerobic bacteria that produce catalase enzyme.

1.2 *Staphylococcus aureus*

In 1884 Anton J. Rosenbach (1842-1923), a German surgeon, isolated two strains of staphylococci, which he named according to the pigmented appearance of their colonies: *Staphylococcus aureus* (Latin aurum, "gold") (Figure 1.1), and *Staphylococcus albus* (Latin albus, "white") (later called *epidermidis*). The genus *Staphylococcus* contains 71 identified species (http://www.bacterio.cict.fr/), which includes pathogens and non-pathogens. Rosenbach showed that *S. aureus* caused wound infections and furunculosis, while *S. epidermidis* was a natural part of the skin flora [2]. *S. aureus* produces coagulase, an enzyme that coagulates the host's plasma by activating prothrombin, which is diagnostically important for differentiating *S. aureus* from other coagulase-negative staphylococci.

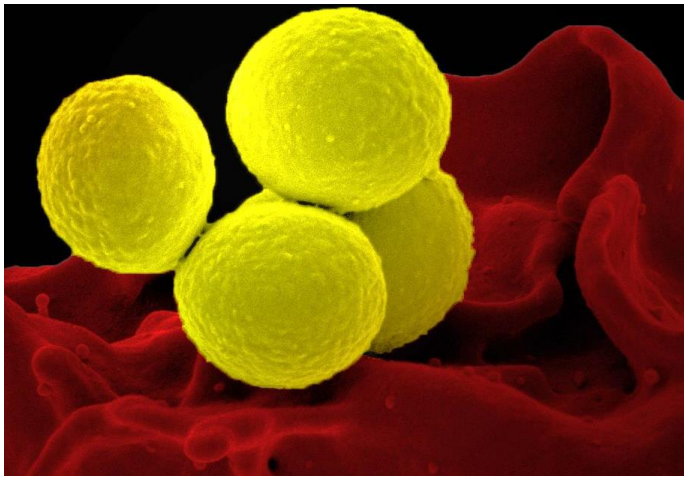


Figure 1.1. *Staphylococcus aureus* (yellow) and human neutrophil cell (red). Courtesy: National Institute of Allergy and Infectious Diseases.

S. aureus colonises the anterior nares of approximately 25 - 30% of the healthy human population [3]. Although a large number of humans are colonised with *S. aureus*, only a little fraction of them develop an infection. Hence, *S. aureus* is comparatively harmless commensal bacteria (facultative pathogen) unless it enters the body through a cut in the skin, and then overcomes the host's immune system. Nevertheless, *S. aureus* is a common cause of skin and soft tissue infections such as abscesses, folliculitis and post-operative wound infections [4]. Previous studies reported that persistent carriers of *S. aureus* are at higher risk for developing endogenous bacteraemia compared with the non-carriers [5].

1.3 *Staphylococcus aureus* and the waves of antibiotic resistance

The development of antibiotics was one of the greatest medical advances for all humankind. Antibiotics were considered as miracle drugs, which eradicated the fear of death due to bacterial infections. In the early 1940s, the introduction of penicillin (a β-lactam antibiotic) into clinical use brought the major infections under control; however, penicillin-resistant Staphylococci isolates were reported two years later [6]. This resistance to penicillin is mediated by penicillinase (β-lactamase) enzyme encoded by the *blaZ* gene, which cleaves the β-lactam ring of the penicillin molecule leading to its ineffectiveness. By the late 1960s, reports showed that more than 80% of staphylococcal isolates were resistant to penicillin [7]. To overcome the effect of

penicillinase, a semisynthetic antibiotic, methicillin, was introduced into clinical practice. Similarly, the launch of methicillin was rapidly followed by reports of methicillin-resistant *S. aureus* (MRSA) isolates [8–10]. Since then, the numbers of MRSA isolates have frequently increased worldwide [11,12]. Hence, new antibiotics, such as gentamicin, vancomycin and ceftaroline were developed and used to treat MRSA infections [13]. However, a similar path of bacterial resistance events has occurred with the introduction of novel antibiotics in clinical practice (Figure 1.2; [14]).

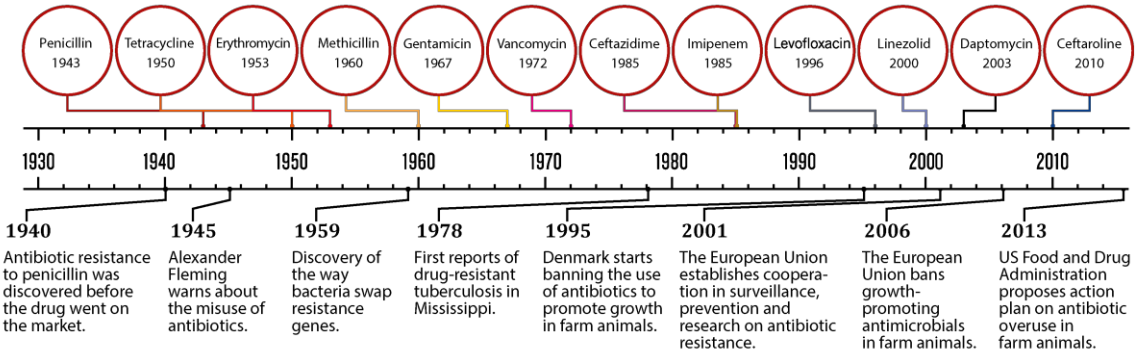


Figure 1.2. The timeline of antibiotic discovery, introduction and overuse. Figure adapted by the author from Hede, 2014 [14].

1.4 *Staphylococcus aureus* genome structure

The first *S. aureus* genome was sequenced and reported in 2001 (Kuroda M, 2001). The *S. aureus* genome is approximately 2.8 Mega-base (Mb) pairs in size with nearly 2,600 open reading frames and has a G+C content of approximately 33% [15]. To date, approximately 4175 *S. aureus* genomes are available on the National Centre for Biotechnology Information (NCBI). The comparison of this huge sequence data revealed that the *S. aureus* genome structure could be divided into two main components: core genome (genetic backbone), and the accessory genome.

1.4.1 Core genome

The core genome is the genetic backbone for all *S. aureus* strains. This core genome constitutes ≈ 75% of the *S. aureus* genome, and encodes housekeeping genes that are highly conserved among all *S. aureus* strains and essential for the growth and

survival of *S. aureus* [15–17]. In contrast to the core genome, the core variable genome is a lineage specific and carries more than 700 variable genes. These genes encode surface binding proteins, virulence factors and toxins, which are known to interact with the hosts' immune system [15,17]. The sequence variation in the core genome is due to either single nucleotide polymorphisms (SNPs) or large variation that range from few nucleotides to several kilo-base (kb) pairs (insertions or deletions of repetitive elements) [15].

1.4.2 Accessory genome

The accessory genome constitutes ≈ 25% of the *S. aureus* genome and is composed mainly of mobile genetic elements (MGEs) that vary among the different *S. aureus* strains. These MGEs are DNA fragments that can be moved between different bacteria [16,17]. MGEs are known to carry genes encoded for antimicrobial resistant, heavy metal resistance, virulence determinants and host adaptation factors [18]. The MGEs have G+C content comparable to the chromosomal DNA, suggesting that the various *S. aureus* lineages acquired the MGEs via horizontal gene transfer [19,20]. *S. aureus* can comprise several types of MGEs, including the staphylococcal cassette chromosome elements, plasmids, transposons, bacteriophages, and pathogenicity islands.

1.4.2.1 Staphylococcal cassette chromosome mec

The characteristic feature of MRSA strains is the acquisition of a mobile genetic element called the staphylococcal cassette chromosome *mec* (SCC*mec*). This SCC*mec* element consists of two essential components; the *mec* gene complex, which is considered as the essential determinant for broad-spectrum β-lactam antibiotics resistance; and the cassette chromosome recombinase (*ccr*) gene complex, which is involved in the mobilization and integration of SCC*mec* element into the *S. aureus* chromosome [21]. The *mec* gene complex carries *mecA* gene and genes that regulate its expression (*mecR1* (promoter) and *mecI* (repressor)). The *mecA* gene (2.1 kb) encodes the penicillin-binding protein (PBP2a or PBP2') that has low affinity for penicillin and other β-lactam antibiotics [22,23]. The *ccr* gene complex comprises of seven to eight open reading frames (ORFs) and the *ccr* gene is placed in the core of this gene complex. Up to the present time, three different *ccr* genes have been reported (*ccrA*, *ccrB*, and *ccrC*) [24]. In contrast to the high similarity of the *mecA* genes, the *ccr* genes

are greatly diverse among staphylococcal species. In addition to the *mec* and *ccr* genes complexes, SCC*mec* element contains so-called joining regions (J-regions), which were classified into J1, J2, and J3. These J-regions may carry additional antimicrobial resistance or virulence determinants [24,25]. J1-3 regions are highly diverse, and their sequences are used for the subtyping of various SCC*mec* types [24,25].

SCC*mec* elements have been classified into distinct types and subtypes based on the combination of the *ccr* gene complex type and the class of the *mec* gene complex. To date, eleven different SCC*mec* types were identified (Figure 1.3) [21,26–33]. The size of SCC*mec* types IV and V (≈24 kb and ≈28 kb, respectively) is comparable, and they are the smallest among the remaining SCC*mec* types [27,28]. However, SCC*mec* IV carries only the recombinase, regulatory, and structural genes for the methicillin resistance, and it lacks the restriction-modification system compared to SCC*mec* V [34]. Hence, SCC*mec* IV may have a lower cost on fitness; therefore, it is frequently acquired by various *S. aureus* lineages such as ST22, which is a major cause of healthcare-associated infections worldwide [27,28,34,35].

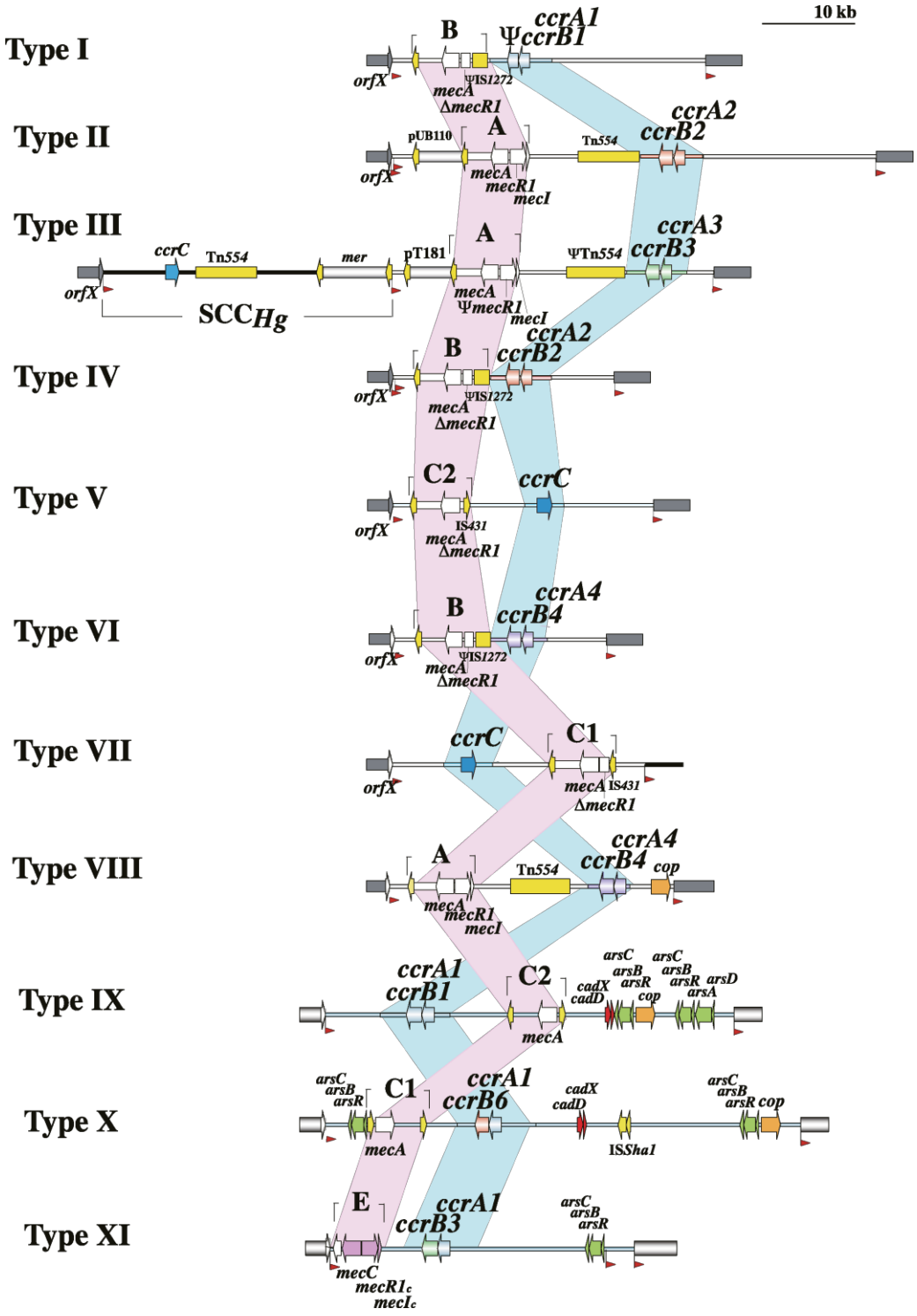


Figure 1.3. The structures of the eleven identified SCC*mec* element types. The pink and blue belts indicate the location of *mec*- and *ccr*-gene complexes, respectively. Figure adapted by the author from Hiramatsu et al., 2013 [24].

1.4.2.2 Plasmids

Plasmids are typically circular DNA molecules, which often carry genes that are beneficial for bacterial survival, including genes that confer resistance to various antibiotic classes such as aminoglycosides, β-lactam and macrolides. In addition, genes encoded for heavy metal resistance and virulence factors such as exfoliative toxin B are frequently found on plasmids [36,37]. *S. aureus* strains can carry up to several plasmids that vary in their gene content and functions.

1.4.2.3 Bacteriophages

Bacteriophages (or so-called phages for short) are viruses that infect bacteria. They have a narrow host range, and their global population was estimated to be on the order of 10^{31} [38]. Bacteriophages attach to specific receptors on the surface of bacteria and inject their phage DNA into the host cell. They are classified into two main distinctive groups: virulent (lytic) and temperate. Virulent (lytic) phages drive the host cell to produce and assemble new phage particles, followed by bacterial cell lyses due to the release of progeny phages. The majority of the phage population is temperate phages, which typically establish a long-term relationship with the host cell. Temperate phages DNA were found to integrate into specific sites of the *S. aureus* genome as prophage and play a significant role in its diversity and evolution [39]. Prophages can affect the pathogenicity of *S. aureus* either by carrying additional virulence factors or by interfering chromosomal virulence genes [39]. Several virulence factors encoded by prophages have been previously characterized, such as the immune evasion cluster (IEC), which encodes the immune-modulating proteins staphylokinase (*sak*), the chemotaxis inhibitory protein (*chip*), the staphylococcal complement inhibitor (*scin*) [40–46]. Furthermore, other prophages encode virulence molecules that have a destructive effect on the human white blood cells, such as enterotoxins and Panton-Valentine leukocidin (PVL) [47].

1.4.2.4 Pathogenicity islands

The *S. aureus* pathogenicity islands (SaPIs) are type of MGEs, which are ≈ 14-17 kb in size and encode additional superantigens and virulence genes [48]. In *S. aureus*, several SaPIs were detected and have been sequenced [48,49]. Integrase, replication and terminase genes are the common features among all detected SaPIs [49,50]. The dissemination of SaPIs among various *S. aureus* lineages occurs via a

helper phage and horizontal gene transfers [51]. Certain *S. aureus* strains have adapted to their ruminant niche by encoding coagulation factor, which was carried by a certain SaPI family [52].

1.5 *Staphylococcus aureus* virulence factors

S. aureus has a broad range of virulence factors that enhance its pathogenicity. *S. aureus* carries several surface proteins that are covalently attached to the peptidoglycan layer (Figure 1.4). These surface proteins, or so-called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), mediate the adherence of *S. aureus* to the host tissue; therefore, they play an important role in the colonization and infection process [53–56]. For instance, (1) staphylococcus protein A binds the fragment crystallisable region of the host's immunoglobulin G (IgG) antibodies and prevents the opsonisation; (2) fibronectin-binding proteins are common adhesins of *S. aureus* strains that contribute to the adherence of *S. aureus* to the host plasma clots; (3) the collagen-binding protein arbitrates bacterial adherence to collagen substrates and collagenous tissues; (4) clumping factors that mediate adherence of bacteria to immobilized fibrinogen and blood clots [54–56].

As soon as *S. aureus* adheres to the host tissues, it is able to escape the host defence mechanisms in several ways, such as biofilm and capsule formation [57–59]. In addition, *S. aureus* can form small-colony variants that hide in the host cells and are relatively protected from antibiotics [60–62]. During infection, *S. aureus* strains produce various numbers of enzymes, such as lipases, proteases, and elastases, which are able to destroy the host tissues [63–66]. Furthermore, some *S. aureus* strains secrete toxins (exfoliative toxins, α-toxins) that facilitate their spread among host tissues (Figure 1.4).

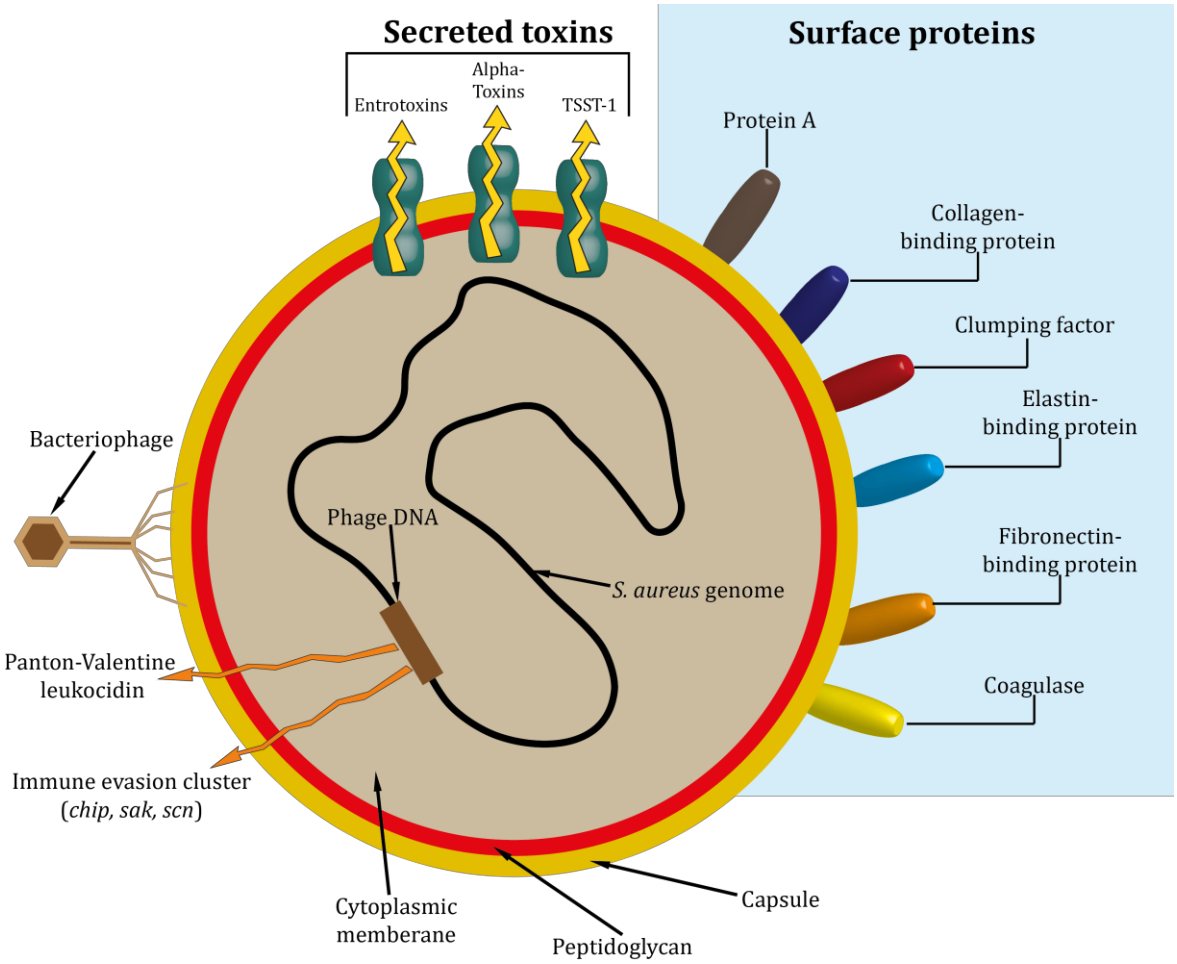


Figure 1.4. Virulence determinants of *Staphylococcus aureus*. TSST-1, toxic shock syndrome toxin 1; *chip*, chemotaxis inhibitory protein; *sak*, immune-modulating proteins staphylokinase; *scin*, staphylococcal complement inhibitor. Figure created by author and adapted from Gordon et al., 2008 [55].

1.6 *Staphylococcus aureus* and the host's innate immune system

Humans and animals are frequently exposed to the invasion of pathogens and have developed systems of immune defence to eliminate infective pathogens in their body. The immune system is comprised of two main parts: innate and acquired immunity. The innate immune system is the first line of host defence against pathogens and is mediated by phagocytes including neutrophils and macrophages [67,68]. Acquired immunity is involved in the eradication of pathogens in the late phase of infection, and the generation of immunological memory. Neutrophils are the first cells recruited from the bloodstream to sites of infection [69–72]. In healthy adult humans, the bone marrow produces, in the steady state, approximately 1 to 2 x 10^{11} neutrophils daily [72].

When *S. aureus* breach the host's skin and mucous surfaces, it is challenged by the host's innate immune system. The *S. aureus* infection stimulates a strong inflammatory response, comprising the migration of neutrophils to the site of infection. Hence, *S. aureus* has evolved mechanisms to resist this frontline of host's neutrophils such as hiding from recognition by neutrophils, blocking the phagocyte receptors or lysing the phagocytes (Figure 1.5). Some of these mechanisms are mentioned in the previous section (1.5), including the capsule and biofilm formation. Additionally, some *S. aureus* strains acquired a prophage, by horizontal gene transfer, that encodes PVL toxin [73]. PVL is known to lyse neutrophils, which are attempting to engulf and kill *S. aureus*, by forming pores in its cell membranes (Figure 1.5) [74–77]. Another important mobile genetic element that could be acquired by *S. aureus*, is the phage φSa3 that carries genes encoding human specific immune-modulating proteins (*chip*, *scin* and *sak*) [40,41,43,78]. These inhibitory proteins help *S. aureus* to prevent the human neutrophils response and phagocytosis [79].

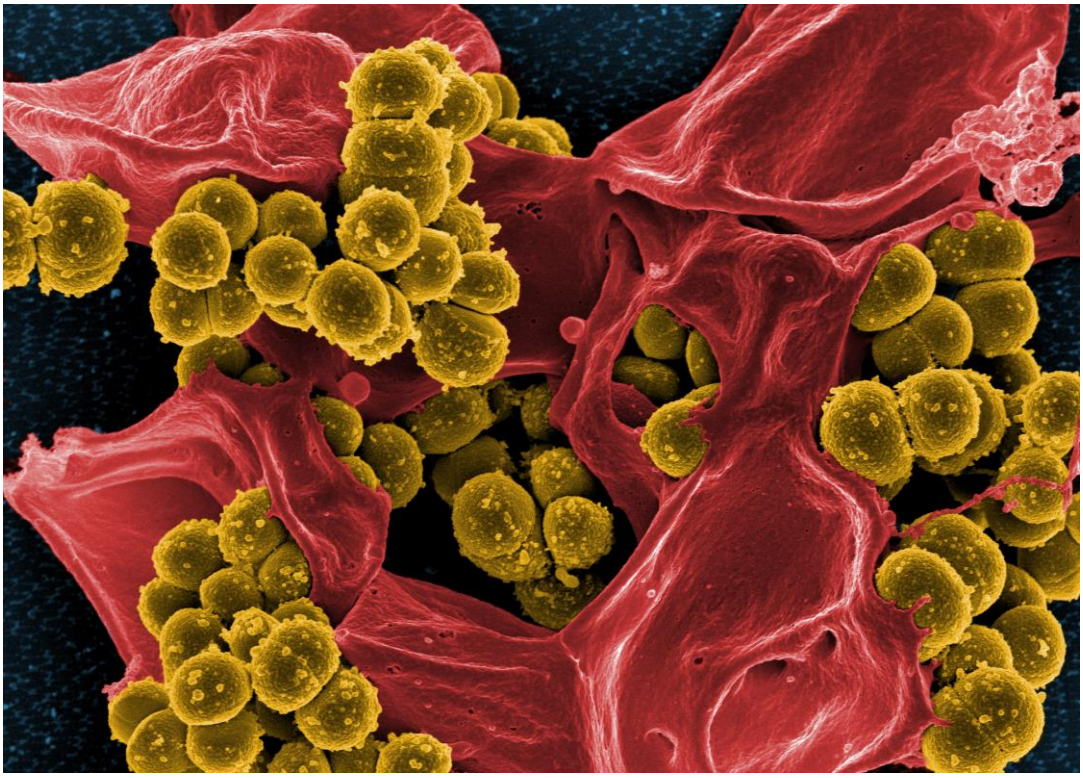


Figure 1.5. *Staphylococcus aureus* (yellow) escaping phagocytosis by lysing the human neutrophils (red). Courtesy: National Institute of Allergy and Infectious Diseases.

1.7 Molecular typing of *Staphylococcus aureus*

Typing refers to the identification of different bacterial strain within a certain species. *S. aureus* typing is the essential key for epidemiological studies, outbreak analysis and infection control. *S. aureus* can be typed either by phenotypic or molecular methods. For several years, phenotypic typing methods such as observing the colony morphology, biochemical reactions, toxin production, antibiotic susceptibility (antibiogram) and bacteriophage typing have been used. However, the poor discriminatory power, the lack of reproducibility, time consuming and the high proportion of non-typable isolates are the main disadvantages of phenotypic typing of *S. aureus*. Therefore, these phenotyping methods were either replaced by or combined with molecular genotyping typing methods. Genotyping classifies the bacterial strains based on distinctive genetic "fingerprints" patterns [80].

1.7.1 Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) was developed in 1984 for the karyotyping and separation of yeast DNA [81]. PFGE is based on digestion of bacterial DNA using rare-cutting restriction enzymes (macro-restriction) that recognize certain endonuclease cleavage sites, which are distributed over the bacterial genome. In 1987, PFGE approach was used for the genotyping of *Escherichia coli* [82,83]. In the 1990s, PFGE has been considered as the "gold standard" among the molecular typing methods for *S. aureus* typing [84,85]. The DNA fragments resulting from the digestion of *S. aureus* genome with rare-cutting enzymes are 10-800 kb in length and can be separated on agarose gels under "pulsed-field" electrophoresis condition in which the polarity of the electric field varies periodically. Subsequently, the separated DNA fragments form a particular band pattern for each *S. aureus* lineage. In contrast to phage typing, PFGE provides improved reproducibility and high discriminatory power, allowing successful investigations of different *S. aureus* outbreaks. However, results inconsistency between different laboratories and interpretation struggles are the main disadvantage of PFGE [86,87].

1.7.2 Multilocus sequence typing

Multilocus sequence typing (MLST) is a sequence based genotyping method, which was introduced, in 2000, for the unambiguous characterisation of *S. aureus* [88]. MLST involves the amplification and sequencing of seven housekeeping genes (each locus ≈ 450–500 bp in length) in the *S. aureus* genome [88]. For each locus, the sequences are compared to previously identified alleles via the MLST website (http://www.mlst.net) and hence a discriminatory allelic profile (seven integers), so-called the sequence type (ST), for each bacterial isolate can be described. Using the Based Upon Related Sequence Types (BURST) algorithm, related *S. aureus* strains could be grouped to distinct clonal complex (CC) [89]. The major advantage of the MLST typing approach is the ability to compare results obtained from various studies through web-based database. However, the lack of sub-typing within the distinct clones, the high cost and the time consumed for analysis are considered the main disadvantages of the MLST approach.

1.7.3 Staphylococcal protein A typing

Staphylococcal protein A (*spa*) is a *S. aureus* surface protein, which plays a significant role in the evasion of host immune responses by binding to the hosts' immunoglobulin G (IgG) [90]. The *spa* gene contains the conserved fragment crystallisable (Fc) binding domain and the X-region (Figure 1.6). This X-region consists of polymorphic tandem repeat sequences (≈ 24 bp per each repeat). *Spa* typing is based upon sequence analysis of these variable number tandem repeats. The repeat sequences are assigned to certain numeric code. Strains with different repeat sequences are assigned definite *spa* types based on the comparison to an international database (http://spaserver.ridom.de). Similar to MLST typing, *spa* typing provides proper discriminatory power that enables the national and international investigation of *S. aureus* outbreaks [91–93]. However, the most important advantages of *spa* typing over MLST are its cost-effectiveness and simplicity, since it requires sequencing only a single locus. Nevertheless, it was shown that infrequently *spa* typing could provide misleading information, which might be due to recombination events concerning the *spa* locus [92,94].

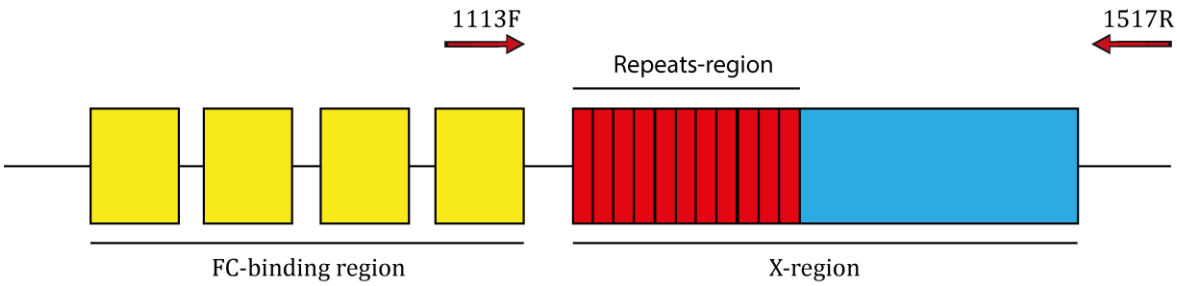


Figure 1.6. Structure of the staphylococcal protein A gene. The fragment crystallisable (Fc) binding domain is represented in yellow colour, while the repeats-region is represented in red colour. Red arrows indicate the annealing sites for the forward 1113F and the reverse 1517R primers.

1.7.4 SCC*mec* typing

The type of the SCC*mec* element can be identified based on the determination of the *mec* complex structure and the different types of *ccr* genes using polymerase chain reaction (PCR)-based approaches. Several approaches were developed till now, however, two PCR methods are commonly used for this determination, either the

multiplex PCR developed by Oliveira et al. that differentiates SCC*mec* type I to IV or the method developed by Ito et al. that involves several PCR assays [26,34,95]. However, both methods showed discrepancies in the results for the same MRSA isolate [96,97]. Hence, another multiplex PCR assay was established to determine the structure of SCC*mec* type I to V [98,99]. In addition, the multiplex PCR assay from Oliveira et al. has been updated to include type V and to improve the detection discriminatory power of SCC*mec* type I to IV [100]. Kondo *et al* developed a convenient system for SCC*mec* typing based on six multiplex PCRs that identify the *ccr* gene complex, the *mec* gene complex and the specific structures in the J regions [101]. However, this method is practicable for routine applications, since the large numbers of required PCR reactions are time consuming [101]. These entire methods share one disadvantages in common that they determine different structural properties of SCC*mec* [102,103]. Hence, a single standardised assay for the determination of the SCC*mec* structure and type need to be developed.

1.8 Population structure of methicillin-resistant *Staphylococcus aureus*

In the 1960s, MRSA was first identified as a healthcare-associated (HA-MRSA) pathogen, which caused nosocomial infections [11]. However, community-associated MRSA (CA-MRSA) infections were detected in human with no prior health care exposure [104].

Previously, the population structure of *S. aureus*, especially MRSA, has been studied using several well-established typing methods, including PFGE [105], MLST combined with SCC*mec* typing [12], single nucleotide polymorphisms (SNPs) analysis [94,106,107] and whole genome sequencing approach [108]. The main findings of these studies could be summarized in as followed (i) the population structure of *S. aureus* is highly clonal [12,89], (ii) the MRSA strains could be assigned to five distinct genotypic lineages [12], and (iii) the SCC*mec* element was introduced on several occasions into different phylogenetic lineages of *S. aureus* [12,20,94,109]. However, in sections 1.8.1 and 1.8.2, below, we review the evolutionary history of both HA-MRSA and CA-MRSA in more details.

1.8.1 Healthcare-associated MRSA

HA-MRSA is highly disseminated in hospitals worldwide [110]. However, the prevalence rate of HA-MRSA varies among the different countries. For instance, the Netherlands and Scandinavia have low HA-MRSA prevalence rates (≤ 1%), while the highest rates (> 50%) were reported in North and South America, Asia and Malta [111–114]. HA-MRSA describes the epidemiological behaviour of certain MRSA strains that are very successful, transfer at higher frequency between patients, and cause epidemic levels of infections in hospitals [115,116].

PFGE technique had been useful for detecting newly emerging MRSA clones only at a local level [117]. Enright et al. applied both MLST and SCC*mec* typing methods on a large international collection of *S. aureus* (912 MSSA and MRSA isolates), and demonstrated that only five CCs (CC5, CC8, CC22, CC30 and CC45) were observed among the MRSA strains [12]. Another study has shown that a predominant epidemic MSSA genotype shared a common MLST allelic profile (ST250) with the first MRSA strains from England, hence, it was suggested that MSSA ST250 is the ancestral genotype of the first MRSA [118]. Later, Robinson et al. demonstrated the multiple emergences of MRSA by proposing that the two MRSA clones (ST247 and ST257) have emerged from the MRSA clone ST250 by stepwise evolution, while the MRSA clone ST254 has emerged independently [119]. Previous studies have reported the impact of recombination events on the evolution of MRSA. For instance, the MRSA ST239 has originated through the acquisition of ≈557 kb and ≈2220 kb chromosomal fragments from ST30 and ST8, respectively [120–122]. The successful dissemination of ST239 in several countries [122,123] may emphasise the influence of recombination events on the emergence of new pandemic clones [121]. Since MLST methods indexes variation at seven housekeeping genes only, it delivers a limited resolution and discriminatory power to elucidate the evolution of different MRSA lineages [106].

Previous studies based on genome-wide SNPs discovery from international MRSA isolate collections demonstrated that the MRSA genomes evolve much faster than previously known (one point mutation every six to eight weeks) [106,108]. Another study based on SNPs analysis revealed that MRSA CC5 lineage has evolved several times through the acquisition of multiple SCC*mec* elements repeatedly into MSSA-CC5 strains [94]. Holden et al. demonstrated that the successful epidemic

spread of EMRSA-15 strains, a HA-MRSA in United Kingdom, is due to mutations that were associated with the evolution of resistance to fluoroquinolones [35]. Similarly, based on whole genome sequencing of a large set of CC30 isolates, McAdam et al. identified the existence of four independent sub-lineages of CC30 [124]. In addition, based on phylogeographic analysis, the authors demonstrated that transmission of CC30 from hospitals in London and Glasgow to regional health-care settings was due to patient transfers [124].

Another study has shown a frequent transmission of the HA-MRSA ST225 clone that had been emerging since two decades in Central Europe [106]. The inter-continental spread and the hospital transmission of MRSA ST239 were demonstrated based on whole genome sequencing approach [108].

1.8.2 Community-associated MRSA

The earliest reported infection cases of community-associated MRSA (CA-MRSA) were caused by a USA400 strain [125]. CA-MRSA clones differ from HA-MRSA by being resistant to a limited number of antibiotics, usually harbour SCC*mec* IV, V or VII and Panton-Valentine leukocidin (PVL) genes [27,110]. The frequent acquisition of the PVL toxin is associated with the tendency for CA-MRSA strains to cause skin, soft tissue and necrotising pneumonia infections [126–128].

Several CA-MRSA clones with different genetic background have emerged in certain parts of the world. For instance, CA-MRSA clone USA300 is the predominant cause of infections in the United States community [129,130]. In addition, this CA-MRSA clone has replaced HA-MRSA strains in North America [131]. In Europe, the PVL positive ST80- SCC*mec* IV (CC80) is the most frequent and widely disseminated CA-MRSA strain [132–134]. The CA-MRSA lineages ST1, ST5, ST9, ST22, ST30 and ST59 were reported in Germany in 2005; however, they have not widely spread in the German community [132].

A previous study based on SNPs analysis revealed that MRSA CC8 has accumulated an increasing number of antibiotic resistances over time [135]. It was suggested that the emergence of CA-MRSA lineages ST8, ST30, ST59 and ST80 is due to inter-continental transmission between the North and South America, Europe, North Africa and East Asia. In addition, it was claimed that ST8 has been spread from the USA to

Europe, while ST80 was transmitted from the Middle East to Europe and then to Asia [136]. A recent study, based on whole genome sequencing revealed that ST80 isolates from Denmark have acquired a novel type SCC*mec* IV with an integrated fractional plasmid, and three integrated prophages encoding PVL and immunomodulatory genes (*sak* and *scn*) [137].

Another study based on comparative genomic analysis of multi-drug resistant ST59 strains, a clone that predominant in Taiwan, has detected truncated *hsdM* and *hsdS* genes that encode the restriction-modification system. Such deficiency in the restriction-modification system may have contributed to the acquisition of mobile genetic elements from enterococci, which confer multi-drug resistance [138]. A recent study, based on genome-wide SNPs discovery has demonstrated that certain sublineages within CC121, a *S. aureus* clone that commonly found as MSSA, were associated with specific clinical phenotypes [107].

1.9 MRSA in animals

In addition to being a human pathogen, *S. aureus* causes a wide spectrum of infections in economically essential livestock and companion animals [139–141]. In 1972, MRSA was reported from cases of bovine mastitis [142]. Since then, reports of MRSA from animals have increased dramatically [141,143–148]. Similar to human, MRSA can colonize the skin and nasal mucosa of healthy animals. Several studies suggested that the animals might act as a potential source of zoonotic MRSA infections in human [149–155].

The population genetic studies have identified genotypes that are associated with specific host species (Figure 1.7). For instance, the MRSA CC1, CC5, CC8 and CC398 were found among ruminants [156,157], while CC1, CC30 and CC398 are frequently found in pigs [141,158]. The MRSA lineages isolated from companion animals tend to reflect the genetic backgrounds of the human MRSA strains [150,159–161], such as CC5, CC8 and CC22 in dogs and cats [152,162] (Figure 1.7). A previous study based on SNPs analysis have revealed that the MRSA CC5 poultry strains have originated in humans and transmitted to poultry, where it subsequently acquired avian-specific MGEs [163]. In contrast, the human pandemic MRSA CC97 strains are de-

scended from bacteria that recently made bovine-to-human jump [164]. Recently, a novel homolog of the *mecA* gene, called *mecC*, has been found among MRSA isolates from bovine mastitis [33]. Afterward, *mecC* have been detected in MRSA strains isolated from livestock, wildlife, and companion animals that originated from several European countries [165–167]. Overall, these studies demonstrated that *S. aureus* clones undergo frequent hosts jumps, and they are able to adapt to novel hosts.

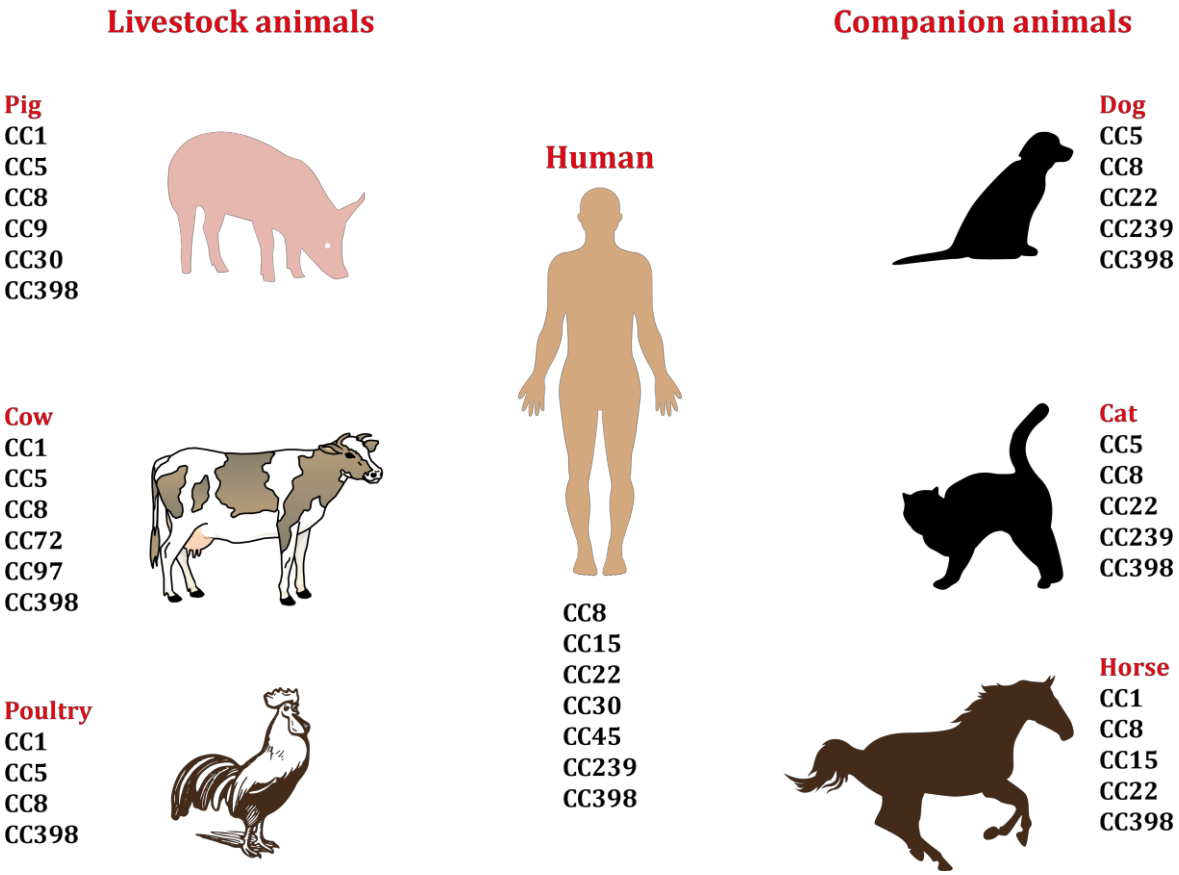


Figure 1.7. The most dominant *Staphylococcus aureus* clonal complexes (CCs) detected in various host species.

1.10 Clonal complex 398

In the early 2000s, MRSA CC398 strains were first detected in France [168], and then in pig farms and farmers in the Netherlands [169]. Since then, MRSA CC398 has been found in pig farms and among their workers in several European countries, North and South America, and Asia [141,145,149,158,170–176]. Hence, the term livestock-associated MRSA (LA-MRSA) was applied to CC398. Although CC398 has been mainly associated with pigs, it has also been found to colonise and cause infections among other host species, including poultry, cattle, and rabbits [141,157,177–179]. Furthermore, CC398 was isolated from horses and small companion animals (cats and dogs) [159,162,180–182]. Although livestock workers are mainly colonised with CC398, transmission from farmers to their family members is infrequent, and decolonisation occurs rapidly after removal of the infection source [149,151,183,184]. However, it was reported that CC398 colonise and cause infections in humans lacking contact with livestock or livestock workers [149,154,155].

MRSA CC398 strains have key genotypic and phenotypic features: first, they are non-typable by PFGE using *SmaI* [185]; second, they harbour SCC*mec* type IVa or V that differ from those carried by other HA-MRSA and CA-MRSA [186]; third, they are resistant to tetracycline macrolides, lincosamides and aminoglycosides antibiotics [187,188]; fourth, they mainly lack toxins such as PVL and enterotoxins [189]; and fifth, they are represented by a large number of *spa* types such as t011, t034, t108, t571, t567, t899, t119, t1451, and t6867 [141].

A Previous study, based on the whole genome sequencing approach, demonstrated that CC398 likely originated in human as MSSA and was transmitted to livestock, where it then acquired methicillin resistance [190]. Furthermore, the same study suggested that CC398 acquired SCC*mec* element in multiple independent events; emphasising the antibiotic selective pressure that occurs in the livestock industry [190]. The descent human MSSA CC398 strains were characterized by *spa* type t571 and they carried the φSa3 prophage (involved in human-specific innate immune evasion), whereas the φSa3 prophage was detected in only one animal isolate [190]. Hence, it was suggested that the φSa3 prophage plays a role in the adaptation of CC398 to the human niche, and its loss was part of the adaptation to non-human

hosts [16,190,191]. Another study revealed that the ancestral CC398 strains (human MSSA CC398 with *spa* type t571) adhere more avidly to human keratinocytes compared with the LA-MRSA CC398 strains, reflecting their wider spread among humans rather than animals [192].

1.11 Aim of this study

This study aimed to the following:

- Elucidating the population structure and the evolution of the *S. aureus* clonal complex CC398.
- To evaluate the adhesion ability of certain CC398 sub-clone to the human and equine fibronectin.
- To investigate the extent to which the innate immune systems of various hosts response to the CC398 infection.
- To determine whether the acquisition of the immune evasion cluster by CC398 leads to its protection from the human's innate immune system response.
- To assess the genomic content of various CC398 strains from different host species based on comparative genomic analysis.

1.12 Study design

This study included CC398 isolates that were partially provided by the German National Reference Centre for Staphylococci and Enterococci at Robert Koch Institute (part of the institute's preserved *S. aureus* isolates collection). Whereas, the remaining CC398 isolates were received from collaborating microbiologists.

The population structure of CC398 was investigated based on mutation discovery using the denaturing high-performance liquid chromatography (dHPLC) approach. Previously, 112 CC398 isolates were analysed using the dHPLC approach by Anne Wittenberg (Table A.1), a former member of Robert Koch Institute, as a part of her doctoral degree in veterinary medicine that was submitted to the Free University of Berlin in January 2014 [193]. To assess whether a certain CC398 sub-lineage had emerged among horses, we extended this isolates collection by including further 83

CC398 isolates that were analysed, in the present study, using the same method (dHPLC) (Table A.1). Furthermore, the association of this CC398 sub-lineage with equine origin was investigated based on in vitro characterisation of host specificity properties and whole genome sequencing approach of representative CC398 isolates.

2 MATERIALS AND METHODS

2.1 Materials

2.1.1 *Staphylococcus aureus* CC398 isolate collection

In this study, a collection of 195 *S. aureus* CC398 isolates was investigated and summarized in Table 2.1. Most of the investigated CC398 isolates were submitted to the German National Reference Centre for Staphylococci and Enterococci at the Robert Koch Institute (RKI), Wernigerode branch, while the remaining isolates were received from international cooperation partner. This CC398 isolate collection was collected between 1993 and 2011; from ten different countries, and 11 various host species. Hence, this collection represented a broad population of CC398. All the investigated isolates details are listed in Table A.1.

Table 2.1. Summary of the *S. aureus* CC398 isolate collection investigated in this study.

Country of origin	Host species	Isolation year	Colonisation/Infection
Austria (n = 17)	Bovine (n = 6)	1993 (n = 1)	Colonisation (n = 29)
Belgium (n = 6)	Cat (n = 1)	2001 (n = 1)	Infection (n = 72)
Canada (n = 1)	Chicken (n = 7)	2002 (n = 2)	Information not available (n = 94)
Denmark (n = 31)	Dog (n = 5)	2003 (n = 3)	
Germany (n = 110)	Environment (n = 1)	2004 (n = 7)	
Italy (n = 3)	Goat (n = 1)	2005 (n = 5)	
The Netherland (n = 15)	Goose (n = 2)	2006 (n = 11)	
Thailand (n = 1)	Horse (n = 53)	2007 (n = 53)	
UK (n = 5)	Human (n = 80)	2008 (n = 31)	
USA (n = 6)	Pig (n = 35)	2009 (n = 33)	
	Turkey (n = 4)	2010 (n = 12)	
		2011 (n = 38)	

2.1.2 Chemicals, enzymes and proteins

All chemicals, enzymes, proteins and DNA markers used in this study are listed below in Table 2.2 and Table 2.3.

Table 2.2. Chemicals, enzymes and proteins used in this study.

Chemicals/Enzymes	Manufacturer
Agarose	Sigma-Aldrich GmbH
Agarose	Life Technologies Corporation
Big Dye® Terminator v3.1 Cycle Seq. Kit	Applied Biosystems®
bisBenzimide H 33342 trihydrochloride	Sigma-Aldrich GmbH
Bovine serum albumin	Sigma-Aldrich GmbH
Bromphenol blue (loading buffer)	Sigma-Aldrich GmbH
Carboxyfluorescein Diacetate Succinimidyl Ester (CFSE)	Sigma-Aldrich GmbH
Distilled water	Sigma-Aldrich GmbH
EDTA (Ethylenediaminetetraacetic acid)	Serva Electrophoresis GmbH
Ethanol (96%)	Merck KGaA
Ethidium Bromide (10mg/mL)	Carl Roth GmbH & Co. KG.
FACSClean	BD Biosciences
FACSFlow	BD Biosciences
FACS Lysing Solution	BD Biosciences
FACSRinse	BD Biosciences
Heparin	Sigma-Aldrich GmbH
HCl (37%)	Carl Roth GmbH & Co. KG.
Horse fibronectin	Biopur AG, Bubendorf, Switzerland
Human fibronectin	Biopur AG, Bubendorf, Switzerland
Lysostaphin	Sigma-Aldrich GmbH
Magnesium chloride (MgCl2)	Carl Roth GmbH & Co. KG.
Optimase Polymerase	Transgenomic, Inc.
Proteinase K	Qiagen GmbH
Sodium chloride (NaCl)	Carl Roth GmbH & Co. KG.
Sodium hydroxide (NaOH)	Carl Roth GmbH & Co. KG.
RNaseI	Sigma-Aldrich GmbH
Taq-Polymerase	Invitrogen, Karlsruhe
Tris	Transgenomic, Inc.
Tris-HCl	Transgenomic, Inc.
Triton X-100	Sigma-Aldrich GmbH
WAVE Puffer A	Transgenomic, Inc.
WAVE Buffer B	Transgenomic, Inc.

WAVE Wash Solution	Transgenomic, Inc.
WAVE Buffer D	Transgenomic, Inc.

Table 2.3. DNA molecular size markers used in this study.

DNA Molecular size markers	Manufacturer
GeneRuler 100bp DNA-Ladder Plus	Thermo Fisher Scientific Inc.
GeneRuler 1kb DNA-Ladder Plus	Thermo Fisher Scientific Inc.

2.1.3 Equipment

All instruments used in this study are listed in Table 2.4.

Table 2.4. Instruments used in this study.

Equipment	Manufacturer
Analytical Balance	Sartorius AG
Autoclave	Biomedis Laborservice GmbH
Biological Safety Cabinet	Thermo Scientific Heraeus®
ChemiDocXR	Bio-Rad Laboratories, Inc
Capillary Sequencer, 3130xl Genetic Analyser	Applied Biosystems, Darmstadt
Centrifuge, Centrifuge 5804R	Eppendorf AG
Centrifuge, Centrifuge 5417R	Eppendorf AG
Centrifuge, MiniSpin® plus	Eppendorf AG
dHPLC WAVETM - System	Transgenomic Inc.
Electrophoresis chamber Sub-Cell GT BioRad	Bio-Rad Laboratories, Inc.
Electrophoresis chamber Power Pac 300 BioRad	Bio-Rad Laboratories, Inc
ELISA plate reader Infinite® M1000 PRO	Tecan Group Ltd.; Männedorf, Switzerland
FACSCalibur	BD Biosciences
Freezer -20. , LIEBHERR comfort	Liebherr-holding GmbH
Fridge + 4., Liebherr Comfort	Liebherr Group
Gel Documentation System GelDoc XR BioRad	Bio-Rad Laboratories, Inc.
Incubator B 6760	Thermo Scientific Heraeus®
Incubator Shakers Innova® 42/42R	New Brunswick Scientific Company, Inc.
Microwave, MW81W	Samsung Group
Multichannel-pipette (8 canals), Research	Eppendorf AG

Pipettes, Reference, variable	Eppendorf AG
Pipettes, Research, variable	Eppendorf AG
Power supply, Power Pack Basic	Bio-Rad Laboratories, Inc.
Pyrosequencer, 454 GS FLX System	Roche Diagnostics GmbH
Spectrophotometer, Biophotometer plus	Eppendorf AG
Thermocycler, GeneAmp PCR System 9700	Applied Biosystems, Darmstadt
Thermocycler, PTC-200 DNA Engine	Bio-Rad Laboratories, Inc.
UV/Vis Spectrophotometer, BioPhotometer plus	Eppendorf AG
Vacuum Centrifuge, Concentrator 5301	Eppendorf AG
Vortex Genie 2	Scientific Industries
Water bath	GFL GmbH
Water purification system	SG Reinstwasser GmbH

2.1.4 Nutrient media

The media used for the cultivation and growth of *S. aureus* isolates are listed in the Table 2.5. The nutrient broths (Becton, Dickinson & Co.) were prepared in the RKI laboratory, Wernigerode branch, while the nutrient agar plates were purchased from Oxoid GmbH.

Table 2.5. Nutrient media used for the cultivation and growth of *S. aureus* isolate collection.

Nutrient media	Reagents	Manufacturer
Mueller Hinton agar (MH) with sheep blood	Tryptone 2%, yeast extract 0.5%, NaCl 10 mM, glucose 20 mM, MgCl2 10 mM, MgSO4 10 mM, sheep blood 5%	Oxoid GmbH
Tryptic Soy Broth (TSB)	Bacto™ Tryptone (Pancreatic Digest of Casein) 1%, yeast extract 0.5%, NaCl 0.5%, glycine (C2H5NO2) 1%, MgCl2 10 mM, pH 7.4	BD Diagnostic Systems
Luria Bertani broth (LB) with 1% glycine	Tryptone 10 g/L, yeast extract 5 g/l, NaCl 0.5 g/L, glycine (C2H5NO2) 1%, pH7.0	Robert Koch Institute

2.1.5 Commercial kits

Commercial kits used in this study are listed below in Table 2.6.

Table 2.6. Commercial kits used in this study.

Kit Name	Usage	Manufacturer
Big Dye® Terminator v3.1 Cycle Seq. Kit	Sequencing the PCR products	Applied Biosystems®
DNAeasy Blood and Tissue kit	DNA extraction	Qiagen GmbH
PCR Master Mix	PCR	Thermo Fisher Scientific Inc.
QIAquick PCR Purification Kit	Purification the PCR products	Qiagen GmbH

2.1.6 Standard solutions

All the standard solutions listed below were prepared in the RKI laboratory, Wernigerode branch.

Phosphate buffered saline (PBS)

NaCl	137 mM
KCl	2.7 mM
Na2HPO4.2 H2O	10 mM
KH2PO4	2 mM
pH 7.4	

Tris-EDTA (TE) buffer

Tris-HCl	200 mM
EDTA	20 mM
pH 7.5	

Tris-Borat-EDTA (TBE) buffer

Tris-HCl	10.78 g
Sodium EDTA (Celaplex III)	0.1 g
Boric acid	5.4 g
pH 8.0	

2.1.7 Software and Internet tools

Table 2.7 represents the software and the web-based tools used for the analysis, manipulation and representation of data.

Table 2.7. Software and web based tools used for the data analysis.

Program	Description	Source
Adobe Suite CS6	Graphic design and photo-editing	© 2014 Adobe Systems Software Ireland Ltd, license Robert Koch Institute
Artemis Release 13.0	Genome browser and annotation tool	http://www.sanger.ac.uk/
Bayesian Tip-Significance testing (BaTs v1.3)	Analysis of phenotype and genotype association with phylogeny	http://evolve.zoo.ox.ac.uk/
BEAST v1.7.5	Bayesian MCMC analysis of molecular sequences	http://beast.bio.ed.ac.uk/
BEAUTi 1.7.5	Generating XML as input data files for BEAST	http://beast.bio.ed.ac.uk/
BioNumerics v6.5	Creating database of loci sequences	Applied Maths, Sint-Martens-Latem, Belgium. License Robert Koch Institute
BRIG v2.3.2	Visualisation of genome comparisons	http://sourceforge.net/projects/brig/
CorelDraw v.12.0.0.536	Graphic design and photo-editing	© 2003 Corel Corporation, license Robert Koch Institute
DnaSP v5.10.01	Analysis of DNA polymorphism	http://www.ub.edu/
EndNote® X5	Reference manager	Thomas Reuters, license Robert Koch Institute
FaBox	Fasta sequences toolbox	http://users-birc.au.dk/biopv/php/fabox/
FigTree v.1.3.1	Graphical viewer of phylogenetic trees	http://tree.bio.ed.ac.uk/
Image Reader v1.5E	Documentation of Agarose gel	Fuji Photo Film

iTOL v2.1	Visualization and manipulation of phylogenetic trees	http://itol.embl.de/
Kodon v3.6.1	Genome alignments and comparisons	Applied Maths, Sint-Martens-Latem, Belgium
Microsoft office package	Office suite	Microsoft
MrBayes v3.1.2	Bayesian analysis	http://mrbayes.sourceforge.net
NavigatorTM software v3.0.0	dHPLC data analysis	Transgenomic Inc., license Robert Koch Institute
NCBI BLAST	Alignment search tool	http://blast.ncbi.nlm.nih.gov/Blast.cgi
PHAST	Identification of prophages	http://phast.wishartlab.com/
R	Statistical computing and graphics	http://www.r-project.org
RAST Server	Genome annotation	http://rast.nmpdr.org
Reverse Complementary	Converting the DNA sequences templates to their complementary	http://www.bioinformatics.org
SeaView v4.2.3	DNA sequence alignment and establishment of ML tree	http://pbil.univ-lyon1.fr/
SeqMan Pro v8.1.5(3), 418	Assembling DNA sequence	DNASTAR Lasergene 8, license Robert-Koch-Institut
Tracer v1.5	Visualizing the BEAST results	http://beast.bio.ed.ac.uk/

2.2 Microbiological methods

2.2.1 Bacterial growth

All the *S. aureus* isolates investigated in this study were streaked onto Mueller-Hinton-Blood agar plates (Oxoid, Cambridge, UK) and cultured overnight at 37 °C. For long-term storage, colonies of each *S. aureus* isolate were inoculated into the cryo-preservative vial, which kept at -80 °C as part of the strains collection of RKI, Wernigerode branch, Germany.

2.2.2 Antimicrobial susceptibility testing

The antimicrobial resistance profile for each *S. aureus* isolate was determined using the broth dilution method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) DIN 58940 guidelines (http://www.eucast.org). The minimum inhibitory concentration (MIC) for the following antibiotics were tested; penicillin G (PEN), oxacillin (OXA), teicoplanin (TPL), vancomycin (VAN), gentamicin (GEN), tetracycline (TET), ciprofloxacin (CIP), moxifloxacin (MFL), trimethoprim / sulfamethoxazole (SXT; cotrimoxazole, and intermediate resistance to SXTi), erythromycin (ERY), clindamycin (CLI), rifampicin (RAM), daptomycin (DAP), mupirocin (MUP), linezolid (LNZ) and tigecycline (TGC).

This test was performed as standard procedure in the National Reference Centre for Staphylococci and Enterococci in RKI, Wernigerode branch, Germany.

2.2.3 Extraction of the chromosomal DNA

For each of the investigated isolates, the chromosomal DNA was extracted using the DNeasy Blood and Tissue kit following the manufacturer instructions, and using 100 µg/ml lysostaphin instate of 20 mg/ml lysozyme.

Briefly, single colony of each *S. aureus* isolate was inoculated in TSB nutrient broth and incubated overnight at 37 °C, with shaking at 150 rpm. From the overnight culture, 1 ml was centrifuged for 5 min at 8,000 rpm and the supernatant was decanted. The pellet was re-suspended in 200 µl lysis buffer (2 mM EDTA, 20 mM Tris-HCl, 1.2% Triton-X-100, 100 µg/ml lysostaphin at pH 8.0) and incubated for 30 min at 37 °C. Subsequently, 25 µl of Proteinase K solution (20 mg/ml) and 200 µl AL buff-

er were added and incubated for another 90 min at 56 °C. To stop the reaction, 200 µl of 96% ethanol were added and the mixture was transferred into DNeasy spin column and centrifuged for 1 min at 8,000 rpm. The spin column was transferred into a new tube and 500 µl AW1 buffer were added, followed by centrifugation at 8,000 rpm for 1 min. Once more, the upper aqueous phase was transferred into a new tube and 500 µl AW2 buffer were added, followed by centrifugation for 3 min at 14,000 rpm. The DNA was eluted from the spin column by adding 100 µl deionized double-distilled water and incubating for 2 min at room temperature, followed by centrifugation at 8,000 rpm for 1 min. The extracted chromosomal DNA was stored at -20 °C.

2.2.4 Quantification of the extracted chromosomal DNA

The concentration and purity of the extracted chromosomal DNA were determined by measuring its absorbance at 260 nm using Biophotometer plus (Eppendorf AG). The DNA concentration of 50 µg/ml was represented by OD260 = 1. The purity of the DNA was determined based on the ratio of absorbance at 260 nm and 280 nm. A ratio of ≈1.8 is usually accepted as "pure" DNA. The presence of protein, RNA or other impurities was indicated with ratio lower than 1.8 and they were absorbed at 280 nm.

2.2.5 Molecular typing of *S. aureus*

2.2.5.1 Spa typing

Spa typing is considered as one of the standardised typing methods for *S. aureus*. *Spa* typing is based on the sequence analysis of the highly polymorphic X region of the *spa* (staphylococcal protein A) locus, which compose of succession of short sequence-variable number of tandem repeats (VNTR) [194]. This VNTR are subjected to point mutations, insertions or deletions that lead to the occurrence of different *spa* types. The *spa* typing method represents a high discriminatory power for outbreak investigations.

Polymerase chain reaction (PCR) was performed to amplify the *spa* repeat region using the forward primer *spa*-1113f (5'- TAA AGA CGA TCC TTC GGT GAG C -3') and the reverse primer *spa*-1514r (5'- CAG CAG TAG TGC CGT TTG CTT -3') [91]. PCR products were sequenced in RKI, sequencing lab using Sanger sequencing method as

described later in paragraph 2.3.2. The software Ridom StaphType™ (Ridom GmbH, Würzburg, Germany) was used for analysing the *spa* sequences of each isolate as described previously [91]. Although *spa* typing does not reach the discriminatory power of *SmaI*-generated macrorestriction patterns, it is widely used for epidemiological typing of *S. aureus* due to comparability and portability of the results.

2.2.5.2 Multi-locus sequence typing

Multi-locus sequence typing (MLST) is an excellent method to investigate the clonal evolution and population structure of *S. aureus*. MLST is based on the sequence analysis of seven relatively conserved housekeeping genes (≈ 500 bp); including carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphatase acetyltransferase (*pta*), triosephonate isomerase (*tpi*) and acetyl coenzyme A acetyltransferase (*ygiL*) [88]. The diverse sequences of each housekeeping gene are assigned as distinct alleles, and these alleles of the seven housekeeping genes form an allelic profile or sequence type (ST). The Based Upon Related Sequence Types (BURST) algorithm groups *S. aureus* strains to defined clonal complex (CC) based on sharing at least five identical loci in common [119,195].

Primers used for amplifying and sequencing these seven housekeeping genes are listed in Table A.2, while the PCR and sequencing conditions are described in paragraphs 2.2.7 and 2.3.2, respectively. For certain isolates, the sequences of the seven loci were assigned and compared with those from all *S. aureus* isolates maintained by the MLST online database (http://saureus.mlst.net/). The disadvantages of MLST typing are that the method remains expensive, laborious and time-consuming.

2.2.5.3 Staphylococcal cassette chromosome mec typing

The *mecA* gene is responsible for transmission of the methicillin and other ß-lactam antibiotic resistance of *S. aureus* and it is located on the mobile genetic element SCC*mec*. Eleven different SCC*mec* types have been described for *S. aureus* based on the type of cassette chromosome recombinases (*ccr*) gene and the class of the *mec* gene complex. SCC*mec* types I to V were typed through a multiplex PCR, while for SCC*mec* IV subtypes an additional simplex PCR was performed as previously described [95]. The primers used for amplifying the *ccr* gene complexes are listed in Table A.3.

2.2.6 Detection of various mobile genetic elements

The PCR approach was used for the screening of certain genes encoded by mobile genetic elements (MGEs) carried by the *S. aureus* isolates. These genes included Panton-Valentine leukocidin (PVL) and the integrase genes (*int*) for the bacteriophages φSa2, φSa3, φSa6 and φAVβ. The PCR primers used for this screening are listed in Table A.4.

2.2.7 Polymerase chain reaction approach

PCR was performed for *spa*, MLST, SCC*mec* typing, and the screening of certain MGEs. A total volume of 50 µl PCR reaction contained 200 µM deoxynucleoside triphosphates (dATP, dCTP, dGTP, and dTTP), 10 pmol of each of the forward and reverse primers, 5 µl 10x PCR reaction buffer (PeqLab, Erlangen), $MgCl_2$ 1.5 mM, and 1.25 U of AmpliTaq DNA polymerase (Applied Biosystems). The amplification reaction was performed in Gene Amp® PCR System 9700 Thermocycler (Applied Biosystems) using the following PCR program: an initial denaturation for 5 min at 80°C, followed by 35 cycles of denaturation for 45 s at 94°C, annealing for 45 s at 60°C, and an extension for 90 s at 72°C, followed by a single final extension step for 10 min at 72°C.

2.3 Mutation discovery

Denaturing high-performance liquid chromatography (dHPLC) is a technique developed for the experimental discovery of single-nucleotide polymorphisms (SNPs) [196–200], and was previously used to elucidate the evolutionary history and population structure of various clonal complexes (CCs) of *S. aureus* [94,106,107,135,163]. The dHPLC allows the automated detection of single base substitutions besides small insertions and deletions, by comparing two or more chromosomal DNA fragments as a mixture of denatured and re-annealed PCR amplicons. The principles behind the SNPs detection through dHPLC is that the heteroduplexes DNA fragments at raised temperature start to melt at the mismatched region and are less thermo stable in comparison with their homoduplexes counterparts. Hence, heteroduplexes elute earlier than homoduplexes and the difference in their retention times indicate the presence of SNP.

In this study, the population structure of *S. aureus* CC398 was investigated based on the mutation discovery at 97 genetic housekeeping loci (≈ 400 bp per locus) in a collection of 195 isolates (112 isolates were analysed previously by Anne Wittenberg, a former member of Robert Koch Institute, while 83 isolates were analysed by the author) (Table A.1). These 97 housekeeping loci were mainly metabolic genes and their polymorphisms provided the most reliable phylogenetic markers for S. aureus [94]. In addition, they were scattered over the core genome of CC398, and constituted 1.4% (40,230 bp) of the CC398 whole genome. In order to reduce the costs and the time consumed for analysis, pools of DNA was created by mixing the DNA from eight to ten different isolates (referred to multiplex DNA), which then used as template to amplify each loci as described below (see paragraph 2.3.1). The PCR products of each locus from these multiplex DNA were compared to their counterparts from the reference strain (08-00301) using dHPLC to detect the heteroduplex. When the chromatographic profile yielded the presence of heteroduplex, then the mutant isolate was identified by simplex test against the reference strain (08-00301).

Directly prior to the dHPLC analysis, the PCR products were denatured by heating at 96 °C for 5 min and then gradually cooled down to 12 °C over a period of 12 min. 5 µl of the PCR products were automatically loaded on the DNAsep HT col-

umn (Transgenomic, Inc., Omaha, USA) and eluted on a linear gradient in WAVE buffer A (0.1M diethylamine acetate, pH 7.0) and WAVE buffer B (0.1 M TEAA, 25% acetonitrile) with a constant flow rate of 0.9 ml/min. The start and end points of the gradient depended on the size of each PCR product. The temperature required for successful resolution of the PCR products was determined for each primer pairs and are listed in Table A.5. Subsequently, UV detector at 254 nm detected the eluted PCR products, and the Navigator™ software was used to analyse the chromatographic profile. The identified SNPs were confirmed by sequencing the PCR products from both ends through capillary Sanger sequencing method using the primers listed in ble A.5.

2.3.1 PCR conditions for the mutation discovery analysis

PCR is considered as the main technique for all high throughput mutational analysis methods. Reliable PCR quality was reached by careful primer design, exclusion of pre-PCR artefacts using "hot-start" methods, optimisation of Mg^{2+} concentration, and prevention of excessive cycle number. In addition, low error rate was achieved by using thermo-stable proofreading polymerases.

The PCR for the dHPLC analysis was performed using a particular Tag polymerase enzyme (Optimase Polymerase) with an efficient 3'→5' exonuclease activity to maximize data reproducibility and to deliver the highest reliability. A total volume of 25 µl PCR reaction contained 2.5 µl 10 x reaction buffer (PeqLab, Erlangen), 16.5 µl deionized double-distilled water, 1 µl forward primer (10 mM), 1 µl reverse primer (10 mM), 2.5 µl dNTP-Mix (each 2 mM dATP, dCTP, dTTP), 1 µl of template DNA (15 ng/µl) and 0.5 µl Optimase Polymerase (2.5 U). The PCR reactions were performed using Gene Amp® PCR System 9700 Thermocycler (Applied Biosystems) with the following program: denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 96 °C for 30 s, annealing at 55 °C for 45 s and extension at 72 °C for 60 s. Subsequently, a final extension step for 5 min at 72 °C, which was followed by cooling at 8 °C.

2.3.2 Capillary-based Sanger sequencing

The Sanger sequencing method was used for the *spa* typing graph 2.2.5.1), MLST typing (paragraph 2.2.5.2) and the confirmation of the SNPs de-

tected by the dHPLC approach. The Sanger method (also referred to as dideoxy sequencing or chain termination) is based on the use of dideoxynucleotide triphosphates (ddNTPs) as DNA chain terminators [201]. Dideoxynucleotides contain a hydrogen group on the 3' carbon instead of a hydroxyl group (OH). When these modified nucleotides integrate into a sequence, then they prevent the addition of further nucleotides and thus the DNA chain is terminated.

The PCR products to be sequenced were purified using the QIAquick PCR Purification Kit (QIAGEN, Crawley, UK), and then amplified using BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, Darmstadt) following the manufacturer instructions. The 10 µl of PCR reaction mixtures were consisted of 8.25 µl nuclease-free water, 1 µl BigDye v3.1; forward and reverse primers 0.5 µl each and 0.25µl of the PCR product. The amplification reaction was performed in Gene Amp® PCR System 9700 Thermocycler (Applied Biosystems) using the following PCR program: denaturation at 96 °C for 5 min, followed by 30 cycles of denaturation at 96 °C for 30 s, annealing at 55 °C for 45 s and extension at 72 °C for 60 s. Finally, an extension step at 72 °C for 5 min and the samples were then cooled to 4 °C. Sequencing process was performed by the sequencing laboratory in RKI, Berlin.

2.3.3 Single-nucleotide polymorphisms analysis

The SNPs discovered by dHPLC were confirmed through the Sanger sequencing method. The software DNASTAR Lasergene 8 (SeqMan Pro) was used for quality assessment and analysing the sequence data. The sequences of the housekeeping loci from each investigated isolate were aligned and compared to their counterparts from the reference strain 08-00301 using SeqMan Pro tool. Subsequently, the identified SNPs by SeqMan Pro were categorised to either ancestral or derived SNPs based on the comparison to previously published *S. aureus* genomes in the National Centre for Biotechnology Information (NCBI) database via the Basic Local Alignment Search Tool (BLAST) algorithm. Afterward, SNPs positions were assigned in both *S. aureus* N315 and CC398 (strain S0385) reference genomes (accession numbers BA000018 and AM990992, respectively). Using the BioNumerics software v6.5 (Applied Maths, Sint-Martens-Latem, Belgium), a database was established to include all identified SNPs, their housekeeping loci sequences, isolates number, and the SNPs positions in both N315 and CC398 genomes.

Based on the identified SNPs the minimum spanning trees (MSTs) were created to study the microevolution of the CC398 isolate collection. For the MSTs a distinct database was established by BioNumerics v6.5, which included all investigated isolates information and their SNPs. In this database, the SNPs were represented in binary code format (presence of the SNP was assigned to 1, while absence of the SNP was assigned to 0). Using the implemented MST algorithm in BioNumerics software, the MSTs were created for plotting the epidemic divergence of CC398 against other factors such as geographical distribution, *spa* types, date of sampling, etc.

2.3.4 Phylogenetic analysis of CC398

A phylogenetic tree is a graphical representation of the inferred evolutionary relationships among entities called taxa that can be genes, collections of genes, or populations based upon comparisons of their sequence data. Several methods of tree construction are used for the phylogenetic analysis, such as maximum likelihood (ML) method. The ML approach estimates the probability that a chosen evolutionary model is most likely explaining the evolution of the observed data. ML trees are based on examining the isolates sequence positions and attempt to create a tree topology that best fits the observed changes to a particular model of evolution.

2.3.4.1 Construction of the maximum likelihood tree

The ML approach was used to estimate the phylogenetic and evolutionary trees from the sequence data of the CC398 isolates. Prior to the creation of ML tree, all the 97 housekeeping loci sequences were concatenated for each of the 195 CC398 investigated isolates (40,230 bp) and aligned using BioNumerics software v6.5 (Applied Maths, Sint-Martens-Latem, Belgium). Reconstruction of the ML tree was performed based on the multiple sequence alignment of concatenated sequences using PhyML software, which is implemented in the multiplatform Seaview v4.2.3 (http://pbil.univ-lypnl.fr/).

Rooting the phylogenetic tree determines its ancestral node and leads to evaluate the direction of evolution. *S. aureus* N315 (CC5, accession numbers BA000018) was assigned as outgroup for rooting the CC398 phylogenetic tree. The correspondent sequences of the investigated 97 housekeeping loci from the N315 genome were concatenated and compared to the sequences of all 195 CC398 isolates. FigTree Software v.1.3.1 (http://tree.bio.ed.ac.uk/) and the online tool iTOL v2.1 (http://itol.embl.de/) were used for visualization and manipulation of the phylogenetic trees.

2.3.5 Molecular evolution of CC398 DNA sequences

Studying the population genetics and molecular evolution of bacteria involve the analysis and comparison of the DNA sequences, to elucidate the diversity/similarity between bacterial populations. Based on this diversity in terms of mutation, selection and genetic drift, models for explaining the evolution process can be

established. In this context, analysis of the multiple alignment of the concatenated sequences from the investigated loci was performed using DnaSP software package (v 5.0). The DnaSP software implements a number of analytical methods allowing effective analysis of the DNA polymorphism from nucleotide sequence datasets. Nucleotide diversity (π), nucleotide variation (θ), synonymous (d_N) and nonsynonymous (d_S) sites, neutrality ($^{d_N}/_{d_S}$), and haplotypes diversity (H) were calculated for the concatenated sequences from the investigated housekeeping loci.

The neutral theory states that the majority of molecular evolutionary changes at the DNA level are driven by neutral drift rather than Darwinian selection. Nucleotide substitutions in protein coding genes can be either synonymous (do not change amino acid; d_N) or nonsynonymous (changes amino acid; d_S). Degree of selection can be estimated by calculating the number of synonymous and nonsynonymous sites, and their ratio. Based on Nei and Gojobori method [202], the total numbers of synonymous and nonsynonymous, S_d and N_d respectively, were calculated as defined by following equations:

$$\boldsymbol{S_d} = \sum_{j=1}^{r} \boldsymbol{s_{dj}}$$

$$N_d = \sum_{j=1}^{r} n_{dj}$$

Where s_{dj} and n_{dj} are the numbers of synonymous and nonsynonymous differences for the codon j, while r is the total number of compared codons. The substitution ratio ($^{d_N}/_{d_S}$) is expected to be less than unity in case of purifying selection, whereas a ratio exceeds unity only under natural selection.

Haplotype diversity (H) is referred to the probability that two random sequences are different, and is defined as

$$H = \frac{n}{n-1}\left(1 - \sum_{i=1}^{h} Pi^2\right)$$

Where *n* represents the number of sequences, *h* is the number of haplotypes, while *pi* is the relative frequency of haplotypes *i*.

Nucleotide diversity (π) is the probability that two random sequences are different at a given site, and was given by

$$\pi = \frac{k}{m}$$

Where *m* is the total number of nucleotide positions and k is the mean number of nucleotide differences. While nucleotide variation (θ) is defined by: $\theta = 4N\mu$, where N is the effective population size, and μ is the mutation rate per nucleotide and per generation [203,204].

2.3.6 Estimating the divergence times based on Bayesian approach

Time to most recent common ancestor (TMRCA) of CC398 was estimated using Bayesian evolutionary analysis by sampling trees (BEAST) software (V 1.7.5) [205]. BEAST software is a Bayesian statistical framework, which provides parameters estimation and hypothesis testing of evolutionary models from the molecular sequence data. The Bayesian phylogenetic inference was established by Thomas Bayes, and is based on Markov chain Monte Carlo (MCMC) algorithms. MCMC is a stochastic algorithm, which relies on sampling theory and considered as the state-of-art method for phylogenetic reconstruction. Metropolis-Hastings MCMC [206,207] is implemented in BEAST as core algorithm to perform the Bayesian analysis. In addition, BEAST focuses on the calibration of the phylogenies by modelling the rate of molecular evolution on each branch in the phylogenetic tree. The molecular clock hypothesis assumes that DNA sequences divergence accumulates approximately with a constant rate over time. This means that the difference between DNA sequences of two bacterial isolates is in proportional to the time of their divergence from their most recent common ancestor (strict model) [208]. On the other hand, BEAST allows phylogenetic inference under relaxed molecular clock models, which do not assume a constant rate across lineages [209,210].

The TMRCA of the investigated CC398 population was estimated using the multiple alignment of the concatenated DNA sequences dataset including the isola-

tion date of each isolate. BEAST software takes input files only in XML format, which describe the dataset to be analysed, the molecular clock models to be used and the chain length of the MCMC algorithm. Therefore, the XML input file was generated from the multiple alignment file (*.fasta) using the Bayesian Evolutionary Analysis Utility (BEAUti) software v1.7.5. Model parameters values were estimated by combining three independent runs, each run with an initial of $5 * 10^7$ MCMC iterations and sampled every 10,000 steps. In addition, the first ten per cent of logged values were discarded as burn-in. Hasegawa-Kishino-Yano (HKY) model was chosen as substitutions model, while *strict clock* and *lognormal relaxed clock* were used independently as molecular clock models with the default settings of *Tree Priors*. The output of the BEAST analysis is a set of tab-delimited text files (*.txt), which included the estimated values and associated effective sample size (ESS) for each model parameter. These output files were analysed by Tracer software v1.5 [205], and all ESS values were greater than 500.

2.3.7 Correlation between phenotypic traits and the phylogeny

Bayesian Tip-association Significance testing (BaTS) software (V 1.0) was used to estimate the correlation between observed phenotypic traits and the phylogeny of CC398. Joe Parker has developed BaTS software to provide a statistical significance test of the null hypothesis that traits are correlated randomly with phylogeny tips [211]. This software is based on three well-established statistics models, the Association Index (AI) [212], Fitch parsimony score (PS) and the maximum exclusive single-state clade size (MC).

The association index statistic model is the sum across all the internal nodes in the phylogeny, and represented by the following model:

$$AI = \sum_{i=1}^{k} \frac{1 - f_i}{2^{m_i - 1}}$$

Here k is the total number of internal nodes, and for each internal node i, f_i represents the frequency of the most common trait value among the tips subtended by that node. m_i is the number of tips subtended by node i. Therefore, low AI values indicate strong association of the observed trait with phylogeny [211,212].

The parsimony score (PS) statistic was calculated using the Fitch parsimony algorithm [213]. The PS statistic for a certain trait takes the range $1 \leq PS \geq n$, where n is the number of tips in the phylogenetic tree. As for AI, low PS scores represent strong association of the trait with phylogeny.

The third statistic model is the monophyletic clade (MC), which was previously applied by Salemi et al [214]. The stronger associations between phylogeny and trait should produce larger monophyletic clades whose tips all share the identical trait. The MC size of certain trait with value x can be defined as followed:

$$MC(x) = {}_{i=1}^{k} max(m_i I_i)$$

Where K represents the number of interior nodes in the phylogeny including the root. m_i is the number of tips sharing the node I and I_i is an indicator function, which equal 1 if all tips sharing the node I have trait value x, and equal zero other-

wise. MC is an integer metric for a single tree and bounded by $1 \leq MC \leq n_x$, where n_x is the tips number that share the trait value x. Association of the phylogeny and trait is positively correlated with MC value.

BaTS integrated the uncertainty arising from phylogenetic error into the analysis using a Bayesian framework. Therefore, the Bayesian MCMC analysis of the sequence data is a precondition for applying significance testing. To exclude significance errors by redundant haplotypes, the significance testing of the observed traits was performed on one haplotype from the multiple haplotypes sharing the same trait in the dataset.

2.4 Phenotypic characterisation of CC398

2.4.1 Phagocytosis assay

The innate immune system is considered as the first line of host's defence against pathogens such as *S. aureus*, which is mediated by phagocytes including macrophages and leukocytes. The phagocytic capacities and activity of different hosts (human, pig and horse) leucocytes (granulocytes, monocytes and lymphocytes) to CC398 were compared flow cytometrically. Briefly, representative CC398 isolates were incubated overnight in Luria Bertani broth (LB) at 37 °C. 1 ml bacterial suspension was washed twice with PBS. 5 and 6- carboxyfluorescein diacetate succinimidyl ester (CFSE) was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 10 mM. Subsequently, bacterial cells were labelled with 1 µl CFSE in 1 ml PBS for 15 min at 37 °C in the dark and then washed three times with PBS before use.

Human whole blood was collected from seven different healthy adult donors (females and males), while pig and horse blood were collected from slaughterhouses from 10 different animals before slaughter. All blood samples were collected into lithium heparin tubes (SARSTEDT AG, Nuembrecht, Germany) and processed within maximum 45 minutes of venepuncture. 35 µl of CFSE labelled bacterial suspension were added to 700 µl of the hosts' whole blood, and incubated at 37 °C with shaking at 1000 rpm. At 5, 30 and 60 min of the start point, 200 µl of the infected blood were transferred into FACS tube with 1 ml FACS lysing solution (BD Biosciences, San Jose, CA), and incubated for 15 min at room temperature. This FACS lysing solution is designed to lyse red blood cells in whole blood and it contained paraformaldehyde for fixing the bacterial cells. Finally, the cells were centrifuged at 450 g for 5 min, and the pellet was suspended in 200 µl FACSFlow™ (BD Biosciences, San Jose, CA).

2.4.1.1 Flow cytometery

The fluorescently labelled bacterial cells were detected with a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) equipped with a 488 nm cooled argon ion laser and 635 nm red-diode laser. Fluorescent bacterial cells were measured at 488 nm (green CFSE), while granulocytes, monocytes and lymphocytes were detected through the side scatter channel (SSC) and assigned as the primary acquisition pa-

rameter. The diverse physical properties of granulocytes, monocytes and lymphocytes allow them to be distinguished from each other and from cellular impurities. The lymphocytes, monocytes and granulocytes have been gated as region 1 (R1), region 2 (R2) and region 3 (R3), respectively.

All parameters were set to linear mode, and the flow rate was set to 12 µl / min. Samples were measured at approximately 3,000 events/s, and a total of 10,000 events were recorded for each cell suspension. Data analysis was performed on the mean fluorescence intensities using Cell Quest software.

This assay was performed in cooperation with the working group of Dr. M. Bischoff at the Institute of Medical Microbiology and Hygiene, Saarland University Hospital; as a part of the BMBF "MedVet-Staph" consortium technical knowledge transfer practices (grant no: D1KI101).

2.4.2 Adherence assay

S. aureus is considered primarily as an extracellular pathogen. Colonisation and invasion of *S. aureus* to different hosts are mediated by the presence of adhesins that bind to components of the extracellular matrix (ECM) of host tissues [54]. Fibronectin (FN), collagen and fibrinogen/fibrin are part of the host ECM, which are targeted by *S. aureus* cell wall anchored surface proteins. Fibronectin is a high-molecular weight glycoprotein, which is involved in cell adhesion, growth, migration and differentiation. Furthermore, it plays a significant role in the mechanism of wound healing. Fibronectin binding proteins (FnBP-A and FnBP-B) were identified as major factors in mediating the uptake of *S. aureus* into host cells [215], and a double mutant FnBP-A/ FnBP-B *S. aureus* strain was not able to colonise the lactating gland in an experimental mouse model of mastitis [216,217].

The adhesion capacity of representative CC398 isolates to the human and equine plasma FN (Biopur AG, Bubendorf, Switzerland) was compared using fluorescence-based approach. High binding black microtiter plates (Greiner Bio-One GmbH, Germany) were coated with 40 µg/ml of either human or equine FN (100 µl/well) diluted in phosphate buffered saline (PBS, pH 7.4) and, subsequently, incubated overnight at 4 °C. The coated microtiter plates were washed twice with 100 µl PBS. Then,

bovine serum albumin (5 mg/ml) was added, and the plates were incubated for 2 h at 37 °C. The plates were washed three times with PBS.

Bacterial isolates were cultured at 37 °C for 3 h in TSB broth. 1 ml of the bacterial suspension was centrifuged at 12,000 g for 1 min, and then the pellet was washed twice with PBS. The pellet was re-suspended in 1 µg/ml of the fluorescent dye bisBenzimide H 33342 trihydrochloride (Sigma-Aldrich GmbH, Germany) and incubated for 15 min at 37 °C in the dark with shaking at 1,000 rpm. Subsequently, the labelled bacterial cells were centrifuged at 12,000 g for 1 min, and then washed twice with PBS. 100 µl of the bacterial cell suspension (OD600 = 1.0 in PBS) were added to each well, and the plates were incubated for 1 h at 37 °C. Plates were washed three times with 100 µl PBS and the absorbance was measured at 465 nm in an ELISA plate reader (Infinite® M1000 PRO, Tecan Group Ltd., Männedorf, Switzerland). This experiment was performed five times for each isolate.

2.5 Whole genome sequencing approach

2.5.1 454 pyrosequencing

The 454 Genome Sequence (GS) platform was the first next generation sequencing platform presented by 454 Life Sciences, which bought later by Roche. This sequencing platform is based on the pyrosequencing approach, which developed by Pal Nyren and Mostafa Ronaghi at the Royal Institute of Technology Stockholm in 1996 [218]. The principle of this method relies on the release of pyrophosphate when a nucleotide is incorporated into a synthesized DNA strand. The released pyrophosphate can be detected through enzymatic reactions in which ATP sulfurylase converts adenosine 5' phosphosulfate (APS) to ATP that enables the second enzyme, luciferase, to react with its substrate (luciferin). This reaction leads to production of visible light, which is measured with a detector (camera). Pyrophosphate will only be released if the DNA synthesis reaction is supplied with the correct dNTP.

The *de novo* whole genome sequencing was performed through Roche/454 FLX platform for six representatives CC398 isolates (Table A.6). The sequencing process was performed at RKI, sequencing laboratory. Briefly, the DNA fragments of suitable size are captured on micron-scale beads (28 μm diameter/each) and amplified in water and oil emulsion, in a process referred to emulsion PCR [219]. Subsequently, the beads containing the coupled DNA fragments are settled into the wells of picotiter plate that contained the enzymatic reagents for the pyrosequencing. The pattern of detected light reveals the nucleotide sequence of each single DNA template. The raw data is represented as a sequence of images, which are normalised, and then transformed into flowgrams. This flowgram is the preliminary data for the sequence analysis [220].

The advantage of 454/Roche GS FLX platform is production of large sequences (approximately 400 bp in size) and the possibility to sequence roughly 1.5 million beads in a single experiment. However, the errors observed for this method are small insertions or deletions (InDels), due to signal-to-noise thresholding problems [221].

2.5.2 Illumina sequencing

Genome sequences of two CC398 isolates recovered from horse infections were received from a cooperation partner at Free University Berlin (FUB) as a part of the BMBF "MedVet-Staph" consortium. In addition, 20 further CC398 genomes were included from a previous study (Table A.6) [190]. These 22 genomes were sequenced using Illumina sequencing technology. Illumina Genome Analyzer platform is based on immobilizing linear sequencing library fragments by adding an Illumina-specific adapter to the ends of DNA molecules. These adapters are found on the oligo-derivatized surface of a sealed glass (8-channels) flow cell. This flow cell allows bridge amplification of the DNA fragments on its surface by using DNA polymerase for producing multiple DNA copies or clusters. Each cluster represents the single molecule that initiated the cluster amplification process.

Illumina/ Solexa adopts the approach of sequencing-by-synthesis, which uses four fluorescently labelled nucleotides simultaneously and removable blocking group to sequence millions of clusters that present on the flow cell surface. These nucleotides carry a base-unique fluorescent label and the 3´-OH group is chemically blocked, which assure the addition of only one base at a time. Therefore, addition of these nucleotides to the template DNA fragment is considered as a unique event. The fluorescent dye released with each nucleotide incorporation event is detected by total internal reflection fluorescence imaging, and then enzymatically cleaved to allow incorporation of the next nucleotide. This series of steps remains for a certain number of cycles, as determined by the user, which provide read lengths of 25-35 bp. A base-calling algorithm assigns sequences and their quality based on signal intensity measurements during each cycle.

2.5.3 Genome assembly

De novo assembly is a method of merging overlapped sequence reads into contiguous sequences (contigs) without the aid of any reference genome as a guide. The six CC398 genome sequences generated through the 454 platform were assembled by the sequencing laboratory at RKI with Roche's GS De Novo Assembler v 2.6 ("Newbler") using default parameters [222], while the two isolates sequenced with Illumina approach the were received from the Free University Berlin were *de novo* assembled

using Velvet [223]. The assembled contigs of each isolate were ordered against the CC398 reference genome (strain S0385; accession number AM990992) using Mauve software version 2.3.1 [224].

2.5.4 Mapping

Mapping refers to comparing and aligning each one of the sequences reads with the reference genome. Prior to the mapping process, the sequences reads files generated by 454 platform were converted from *.Sff to *.Fastq format using an open source tool sff_extract 0.3.0 (http://bioinf.comav.upv.es/sff_extract/download.html). The sequence reads from the 28 genomes in *.FastQ format were mapped against the published CC398 reference genome (strain S0385; accession number AM990992) [225] using the Burrows-Wheeler Alignment tool (BWA), which is implemented in the *mapping_pl* script developed by Matthias Steglich (RKI member).

Kodon build-in function *Repeat and Match Search* was used with default settings to determine the repetitive sequences. In order to avoid false SNPs calls, the SNPs located in the repetitive sequences regions and the mobile genetic elements were excluded using the *Fas_handle (--substitute)* script developed by Matthias Steglich (RKI member). SNPs calling were performed using SAM tools, which is implemented in the *Fas_handle (--snp_filter)* script. Based on the SNPs alignment of all 28 CC398 genomes sequences, a maximum likelihood tree was constructed as described previously in paragraph 2.3.4.1. ST36 (accession number BX571856) was previously determined to be the most closely related non-CC398 STs [190]. Therefore, ST36 was used as outgroup for rooting the CC398 phylogenetic tree, and for identifying the most ancestral CC398 clade.

2.5.5 Genome annotation

The annotation is referred to the process of adding information to the nucleotides sequences of DNA. For genome annotation a wide range of software solutions and annotation platforms are available. Detection of open reading frames (ORFs) and automated annotation of all genome-sequenced isolates were performed through RAST-Server at CeBiTec in the University of Bielefeld, Germany [226]. RAST is a freely accessible server that delivers initial gene calls, gene functions, and an accurate annotation for bacterial genomes. Ordered genome contigs in multi-fasta files format were

uploaded to RAST and compared to the previously published CC398 reference genome (strain S0385; accession number AM990992) to identify the ORFs. The genomes annotation files were downloaded in GenBank format, and were examined using Artemis software (http://www.sanger.ac.uk/) (v13.0).

2.5.6 Comparative genomics

Which genes are unique to these particular genomes and which genes do they share with the reference genome? Do these isolates carry specific genes that are known to play a role in host adaptation, virulence, or drug resistance? In order to answer these questions the comparative genomes analysis was performed. Eight genomes sequences (six from RKI, two from the Free University Berlin; Table A.6) were compared with the CC398 reference genome (strain S0385; accession number AM990992) using Kodon implemented option (DNA comparative chromosome mapping).

BLAST Ring Image Generator (BRIG) is a Java based tool, which was used to visualize the comparison of the reference sequence to the eight genomes sequences as previously described [227].

3 RESULTS

3.1 Molecular typing of CC398 isolate collection

In this study, a collection of 195 *S. aureus* isolates was investigated. These *S. aureus* isolates were collected between 1993 and 2011, from ten different countries (mainly from Europe), and various hosts (humans: n = 80; animals: n = 115) (Table A.1). Molecular typing techniques such as *spa*, SCC*mec* were applied on all isolates.

Fourteen different *spa* types were identified among the 195 CC398, including t011, t034, t108, t571, t799, t899, t1197, t1344, t1451, t2576, t2876, t2974, t5972, and t6867 (Table A.1). The *spa* types t011 (repeat units pattern; 08-16-02-25-34-24-25) and t034 (repeat units pattern; 08-16-02-25-02-25-34-24-25) were the most predominant, representing ≈ 45% (n = 88) and 41% (n = 80) of the entire isolate collection, respectively (Figure 3.1).

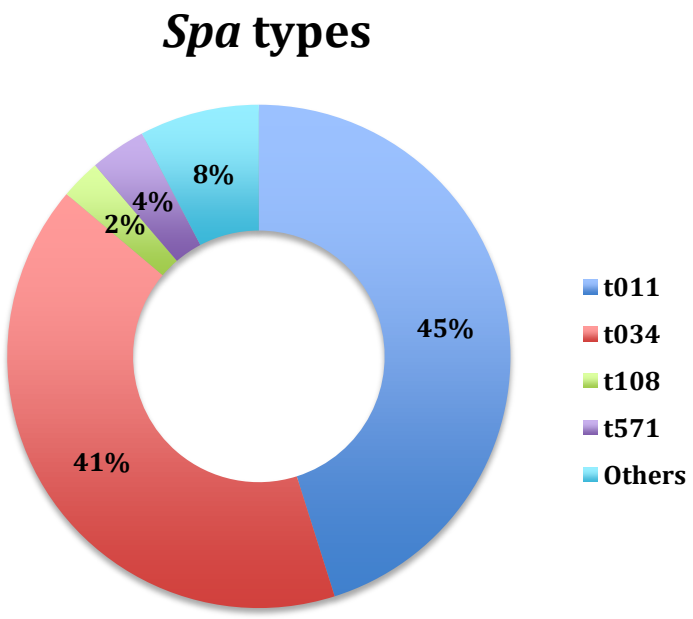


Figure 3.1. Distribution of the various *Spa* types among the investigated CC398 isolates (n = 195).

Among the investigated CC398 isolate collection, 37 isolates were MSSA, while the remained 158 isolates were MRSA (Table A.1). The SCC*mec* typing revealed that approximately 50% of the isolates (n = 99) harboured SCC*mec* type V, while 27% of the isolates (n = 52) acquired SCC*mec* type IV (Figure 3.2).

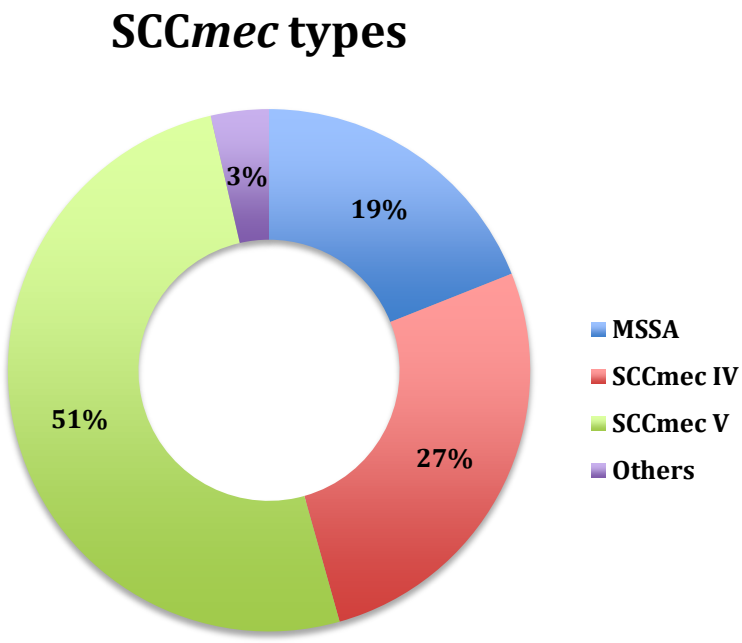


Figure 3.2. Distribution of the different SCC*mec* types among the investigated CC398 isolates (n = 195).

3.2 Antimicrobial resistance phenotypes

Antimicrobial susceptibility testing of the entire CC398 isolate collection (n = 195) displayed 56 different susceptibility patterns (Table A.1). Most of the isolates were resistant to penicillin (n = 187), tetracycline (n = 179) and oxacillin (n =155). All isolates were susceptible to vancomycin, daptomycin, and linezolid.

In total, 18 different antibiotics were included in this susceptibility test. These antibiotics represented the following 12 antibiotic classes penicillins, cephalosporins, carbapenems, monobactams, fluoroquinolones, aminoglycosides, glycopeptides, macrolides, lincosamides, streptogramins, clindamycin, tetracyclines, and miscellaneous. 41% of the isolates were resistant to a range of 1 to 3 different antibiotics classes, while 31% of the isolates were resistant to more than 5 antibiotic classes (Figure 3.3).

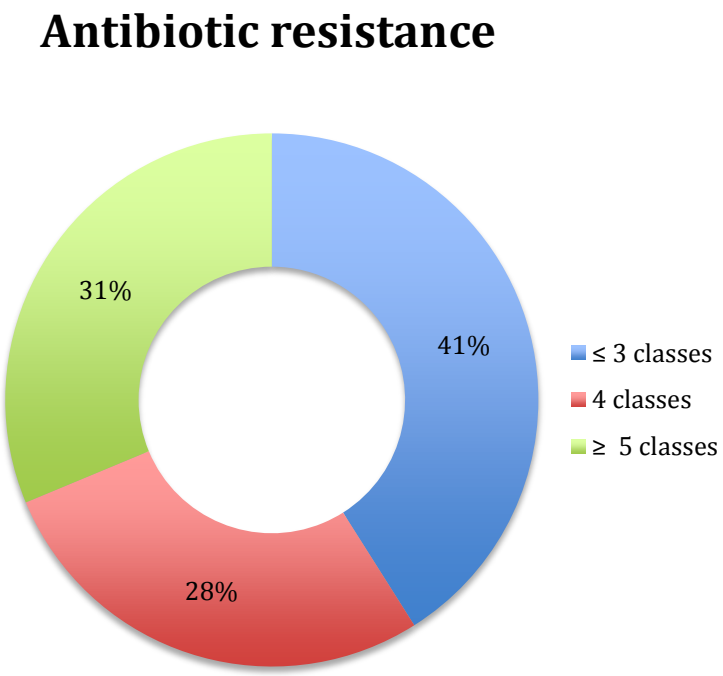


Figure 3.3. Proportion of resistant isolates to different numbers of antibiotic classes.

3.3 Phylogenetic analysis of CC398 based on mutation discovery

The mutation discovery analysis for the CC398 isolate collection was performed through the dHPLC approach. SNPs were detected at 97 genetic housekeeping loci (≈ 400 bp per locus) that are distributed along the *S. aureus* chromosome and are known for their metabolic functions. They constituted 1.4% (40,230) of the CC398 chromosome. These 97 housekeeping loci were amplified for each of the 195 CC398 isolates as described in paragraph 2.3.1 page 34 using the PCR primers listed in Table A.5. The PCR amplicons from each isolate were compared to the reference strain (08-00301) for detecting the heteroduplexes that indicated the presence of SNPs. The identified SNPs were confirmed through capillary Sanger sequencing of the PCR products as described in section 2.3.2 page 34.

For SNPs confirmation, sequences from the suspected loci were aligned to their reference strain counterparts using the software Lasergene v.8 (Figure 3.4). Figure 3.4 illustrates the confirmed SNP in locus au301 for the isolate 09-03343. For assigning the confirmed SNPs to either ancestral or derived SNPs, the correspondent loci sequence had been blasted in the NCBI database and compared against the previously published *S. aureus* genomes. The SNPs positions in both N315 and CC398 (strain S0385) reference genomes (accession numbers BA000018 and AM990992, respectively) were determined as well.

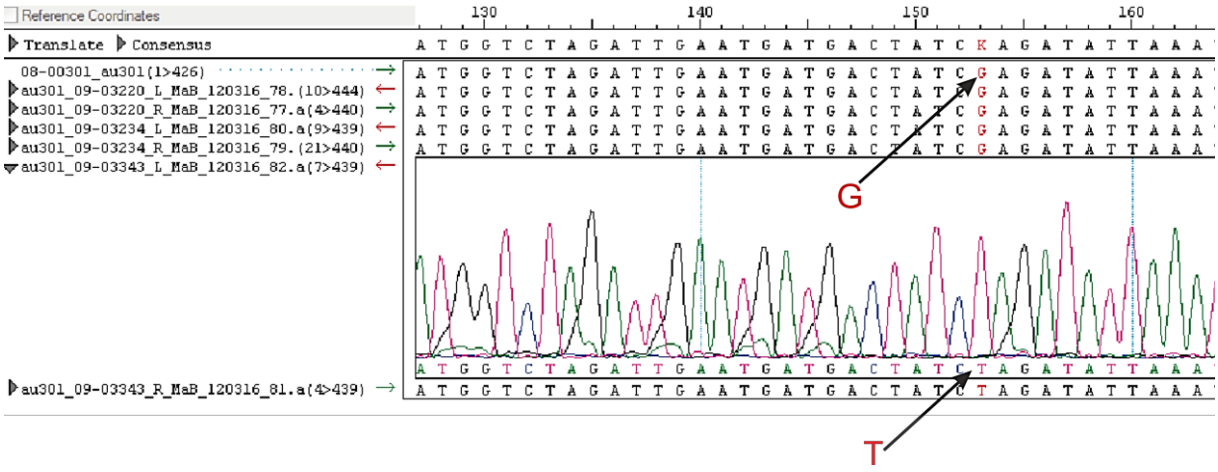


Figure 3.4. Sequences alignment for the locus au301 from different CC398 isolates against the reference strain (08-00301). The detected SNP (G -> T) is represented in red colour.

Based on this analysis, 96 bi-allelic polymorphisms (BiPs) (i.e., polymorphic sites at which two alleles were observed) were detected, which were associated with 63 haplotypes. Among these polymorphisms were 34 synonymous point mutations in the protein coding genes (i.e., substitutions in coding regions that result in the same amino acid), 58 non-synonymous point mutations (i.e., substitutions that lead to change in the amino acid sequence of protein), and 4 insertions or deletions (Indels) ranging in size from 1 to 14 bp (Table A.7). Within these 96 BiPs, 41 polymorphisms were informative for maximum parsimony analysis.

The nucleotide diversity (π, the average number of nucleotide dissimilarities per site among two isolate sequences) for the coding regions was 0.00008 ± 0.00001, while the estimated mean nucleotide substitution rate was 5.4×10^{-6} substitutions/nucleotide site/year (95% confidence interval, 3.5×10^{-6} to 7.5×10^{-6}).

3.3.1 BEAST analysis

The concatenated sequences and their isolation date were used to estimate the time to most recent common ancestor (TMRCA) by applying a Bayesian coalescent approach. Based on the estimated mutation rate, the sequences variations and the isolation date (1993 - 2011) of the isolates dataset, the TMRCA of the 195 CC398 isolates was estimated to be back to ≈ 1974 (95% confidence interval, 1955 to 1991).

3.3.2 Minimum spanning tree

Based on the detected 96 polymorphisms, the minimum spanning tree was constructed using BioNumerics software as described in section 2.3.4 page 37. The minimum spanning tree composed of 63 haplotypes and showed very limited diversity among the 195 investigated CC398 isolates (Figure 3.5). The ancestral node of the phylogenetic tree (*, Root) was determined by comparing the concatenated sequences from the investigated loci of all investigated CC398 isolates with their counterparts of the previously published *S. aureus* N315 (accession numbers BA000018) as an outgroup.

The minimum spanning tree revealed six main clades (A to F) within CC398. Each clade is represented by different CC398 isolates that share at least one SNP in common. The SNPs defining each clade are listed below in Table 3.1.

Table 3.1. Point mutation(s) defining each clade.

Clade	SNP-ID	Quality	Ancestral	Derived	Position in ST398 genome (strain S0385; accession number AM990992)	ORF in ST398 genome	Product in ST398 genome
A	au251-1	Non- Synony-mous	T	A	827455	SAPIG0794	Urea amidolyase
B	au209-1	Non- Synony-mous	C	T	358137	SAPIG0324	Carbohydrate kinase, PfkB family
C	au309-2	Synonymous	A	T	2533404	SAPIG2453	Nitrite reductase [NAD(P)H], large subunit
D	au200-1	Non- Synony-mous	C	T	23542	SAPIG0017	Adenylosuccinate synthetase
	au289-1	Non- Synony-mous	T	G	813835	SAPIG0778	N-acetylglucosamine-6-phosphate deacetylase
E	au291-1	Non- Synony-mous	G	A	1074694	SAPIG1020	5' nucleotidase family protein
F	au261-1	Synonymous	T	C	1929639	SAPIG1818	Proline dehydrogenase

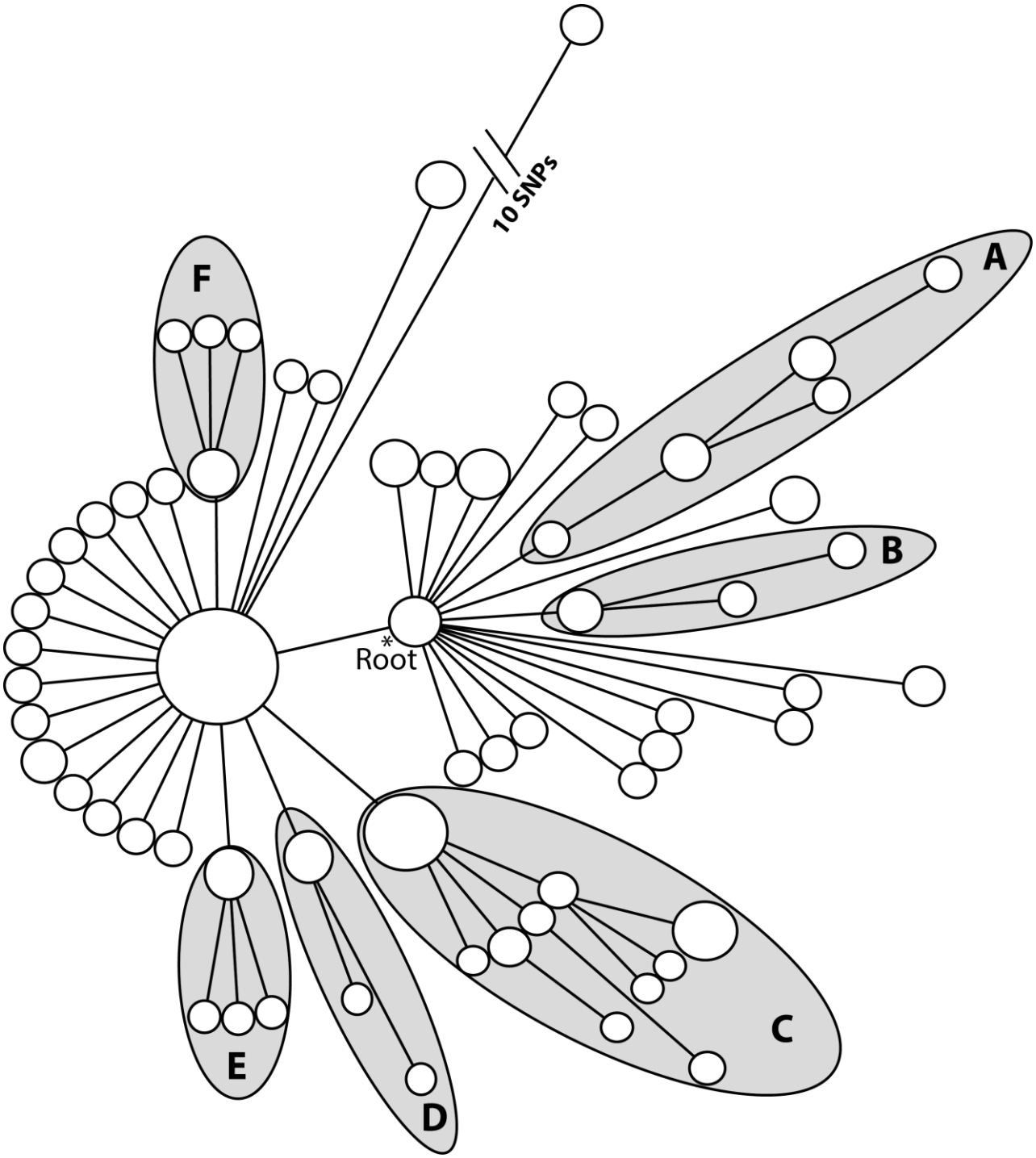


Figure 3.5. Minimum spanning tree of the investigated 195 CC398 isolates based on the 96 identified SNPs. Each circle represent a haplotype and the circles size correspond to the number of isolates sharing the same haplotype. The position of the ancestral node (*, Root) was determined by comparing the concatenated sequences from the investigated loci of all investigated CC398 isolates with the concatenated sequences of the previously published *S. aureus* N315 as an out-group. The minimum spanning tree revealed six main clades (A to F) within CC398.

3.3.3 The Maximum likelihood approach

The maximum likelihood is a tree-building method for inferring the phylogenetic relationships from sequence data. The ML approach was used to predict the evolutionary dynamics of CC398 and to describe the important trends in the data collection without over fitting. The ML model parameters were optimized as described in section 2.3.4.1. In this context, all the 97 housekeeping loci sequences were concatenated for each of the investigated CC398 isolates (40,230 bp), subsequently, a multiple sequence alignment was created, which was used for constructing the ML tree.

The phylogenetic tree of the investigated CC398 isolates (Figure 3.6) revealed identical structure, root position and the six different clades (A-F) as in the MST (Figure 3.5).

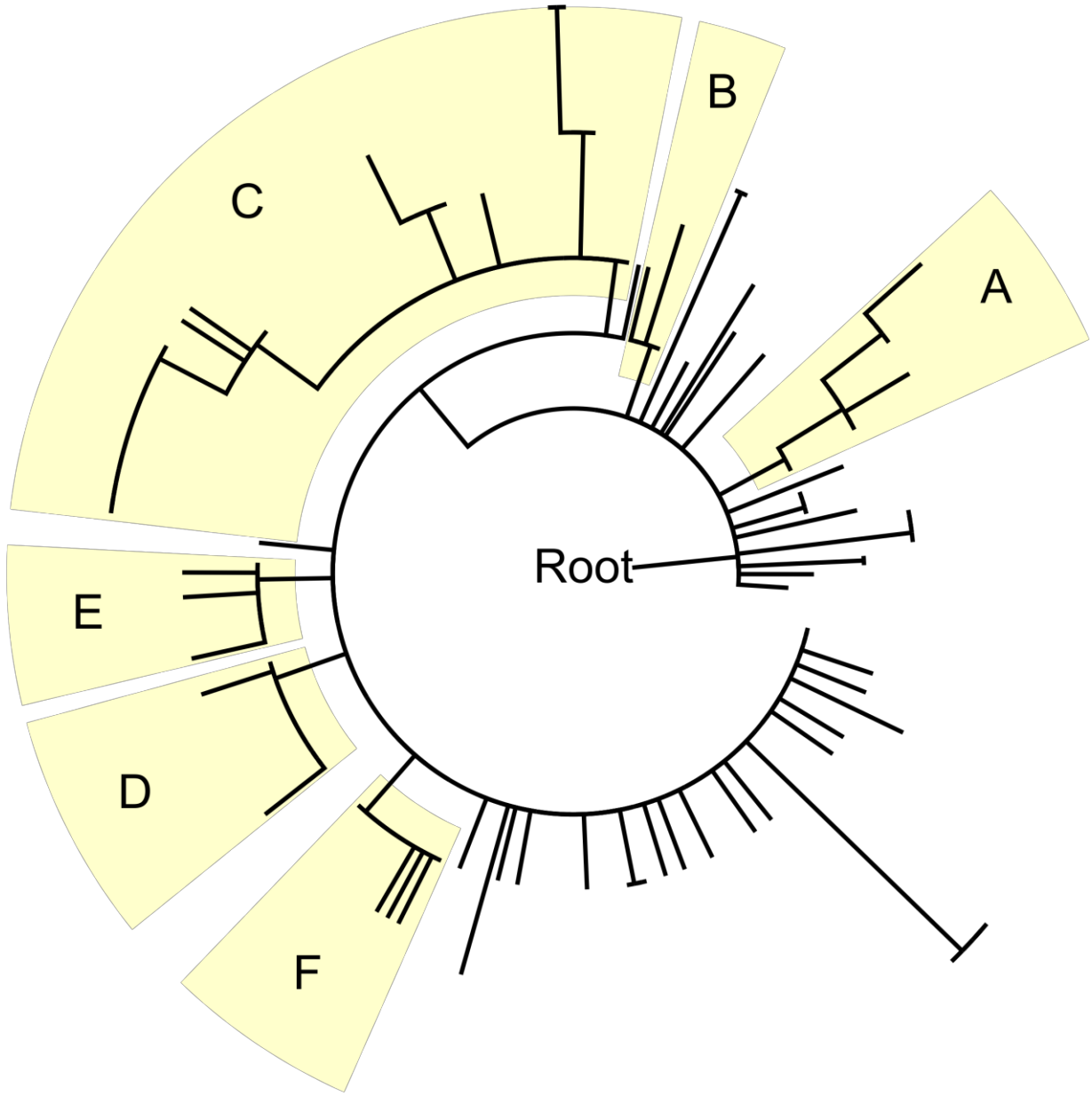


Figure 3.6. Maximum likelihood tree of the 195 investigated CC398 isolates based on 96 polymorphisms.

3.3.3.1 Phylogeny and the geographical distribution of CC398

The geographical distribution of the investigated CC398 isolate collection is represented in Figure 3.7. This CC398 isolate collection represented ten different countries. The countries of origin for the investigated CC398 isolates were plotted on the MST in different colours. Clades A (n = 10), E (n = 9), and D (n = 13) with exception of one isolate (the previously published strain S0385 from the Netherlands in clade D) consisted of isolates that were collected from Germany. In addition, clade F harboured 11 isolates; three of them were collected from Denmark, while the remaining eight isolates were from Germany. Clade B contained five isolates, which were originated from four different countries (Denmark, n =2; Germany, n = 1; Italy, n = 1; the Netherlands, n = 1). Clade C composed of 52 isolates with different geographical backgrounds (Austria, n = 15; Belgium, n = 2; Germany, n = 29; and the Netherlands, n = 6).

BaTS analysis was used to resolve the association of the geographical origin of the investigated CC398 isolates with their phylogeny. The BaTS analysis is summarised in Table 3.2 and revealed that certain countries of origin (Austria, Denmark, Germany, Italy, the Netherlands, and UK) were significantly associated with the phylogenetic traits.

Table 3.2. BaTS analysis results show the association of the geographical distribution of the investigated CC398 isolates with the phylogeny

Country of origin	MC value	p-value
Austria**	13.72	0.009
Belgium	10.39	1
Canada	1	1
Denmark**	31.38	0.009
Germany**	10.58	0.009
Italy**	2	0.009
Thailand	1	1
The Netherlands*	20.39	0.029
UK**	4.06	0.009
USA	14.95	1
AI=8.89; p=0		
PS=59.80;p=0		
*, $p < 0.05$; **, $p < 0.01$		

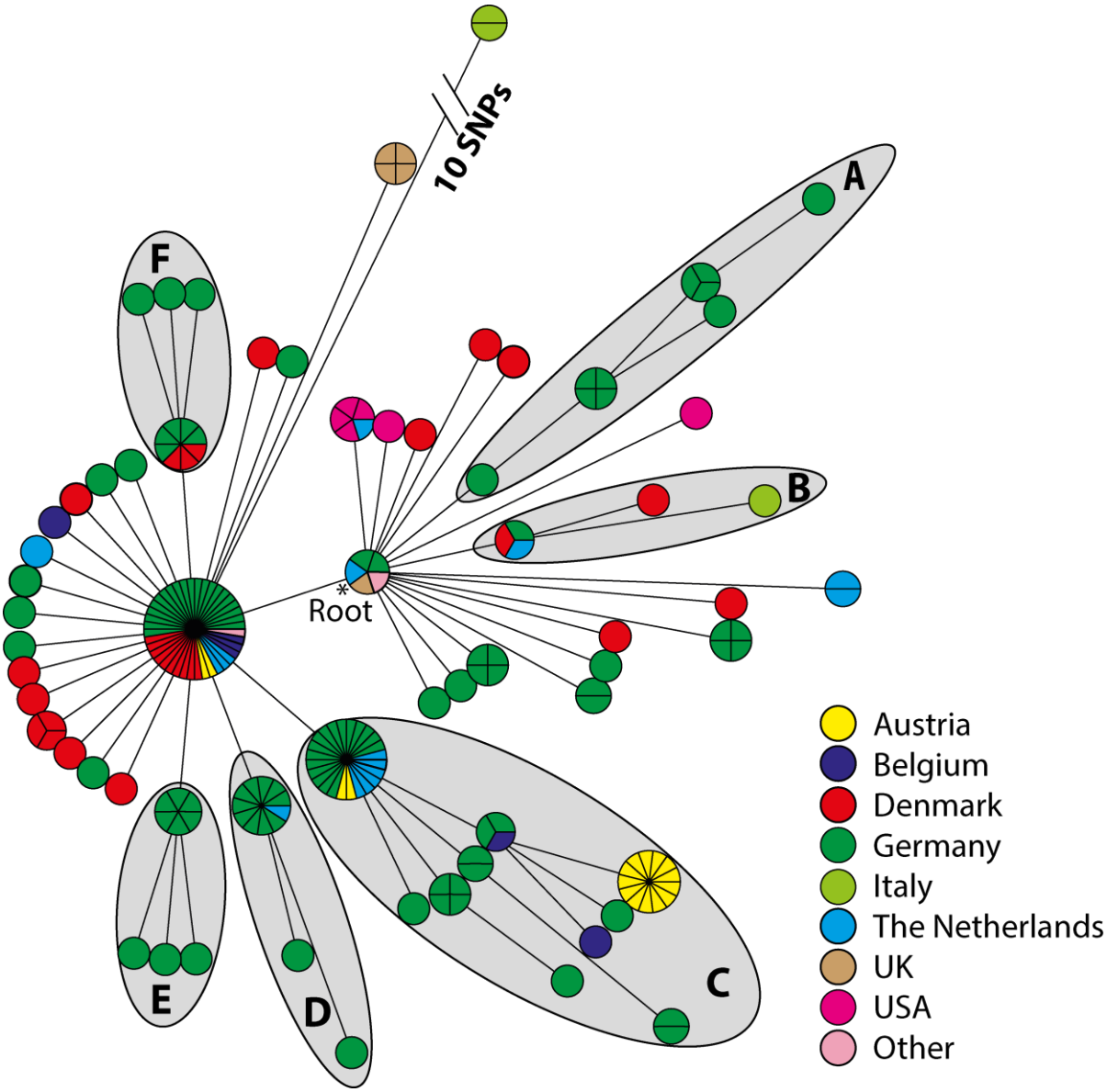


Figure 3.7. Minimum spanning tree represents the geographical distribution of the investigated 195 CC398 isolates. The different colours represent the different countries of origin.

3.3.3.2 Phylogeny and spa types

Mapping the 14 different *spa* types onto the MST shows that clades A (n = 10), E (n = 9) and F (n = 11) consisted of isolates characterised by *spa* type t034, with exception of one isolate within clade F that was represented by *spa* type t011 (Figure 3.8). Similarly, all isolates in clade A (n =5) shared the same *spa* type (t108). Clades D (n = 12) and C (n = 52) comprised isolates characterised by *spa* type t011 (Figure 3.8), with exception of three isolates in clade C (t6867 (n =2) and t779 (n = 1)) (Table A.1).

Interestingly, isolates with *spa* type t899 (n = 2) were the most divergent haplotype in comparison with the remaining CC398 isolates (Figure 3.8). These t899 isolates had ten point of mutations compared to the root (au200-2, au200-3, au201-1, au201-2, au201-3, au202-1, au202-2, au202-3, au202-4 and au202-5). These SNPs were located on the isolates chromosomes within a region of ≥ 111,139 bp (between 23,209 - 134,348) (Table A.7). Hence, the SNPs from these t899 isolates were excluded from the phylogenetic analysis. In total, seven of the investigated CC398 isolates were represented by *spa* type t571; six of them were belonging to the most ancestral lineage of CC398 (Figure 3.8).

BaTS analysis revealed that *spa* types t011, t034, t108, t571, t899, and t1457 were significantly associated with the phylogeny ($p < 0.01$; Table 3.3).

Table 3.3. BaTS analysis results show the association of the investigated CC398 isolates' *spa* types with the phylogeny

Spa-type	MC value	p-value
t011**	1.92	0.009
t034**	10.69	0.009
t108**	5	0.009
t571**	20.49	0.009
t899**	2	0.009
t1197	1	1
t1344	1	1
t1451	1	1
t1457**	2	0.009
t2576	1	1
t2876	1	1
t2974	1	1
t5972	1	1
t6867	1	1
AI=5.74; p=0		
PS = 42; p = 0		
**, $p < 0.01$		

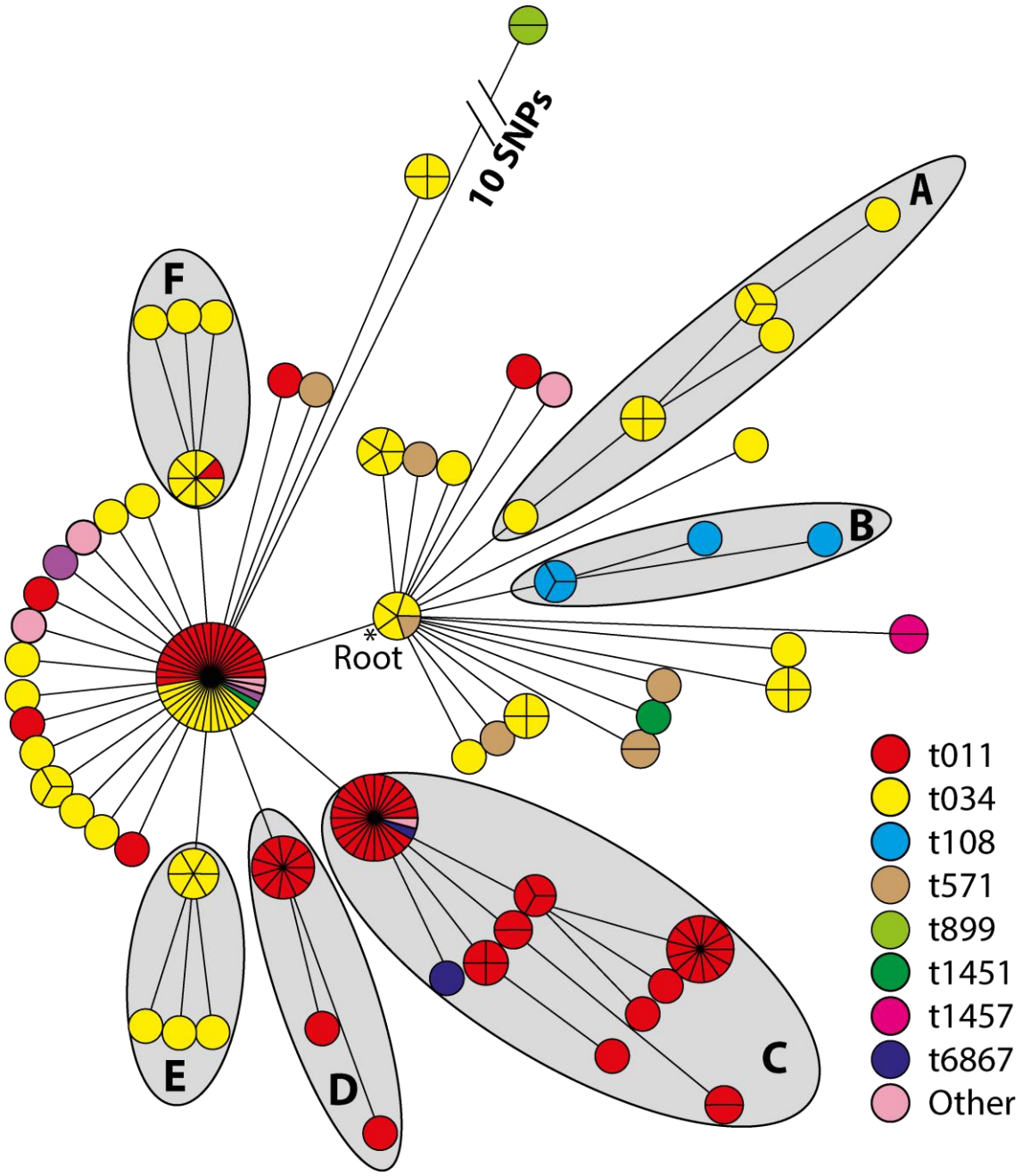


Figure 3.8. Minimum spanning tree shows the various *spa* types that represented the investigated CC398 isolates' collection.

3.3.3.3 Phylogeny and SCCmec types

Figure 3.9 represents the distribution of the detected SCC*mec* types onto the phylogenetic tree of the investigated CC398 isolates. The MST revealed that clades A (with exception of three isolates), B (with exception of one isolate), D, E and F (with exception of three isolates) carried SCC*mec* V. Clade C composed of isolates that harboured SCC*mec* IV with exception of four isolates which had SCC*mec* V. In total, 17 MSSA isolates out of 37 were located in the ancestral clade, while the remained 20 MSSA isolates were distributed across the phylogenetic tree (Figure 3.9).

BaTS analysis demonstrated that the MSSA isolates, and the CC398 isolates with SCC*mec* types IV and V were significantly associated with the phylogeny ($p < 0.01$, Table 3.4).

Table 3.4. BaTS analysis results show the association of the detected SCC*mec* types with the phylogeny

SCC*mec*-type	MC value	p-value
MSSA**	43.96	0.009
II	1	1
III	1	1
IV**	21.43	0.009
V**	13.72	0.009
VII	1	1
IX	1	1
X	1	1
n.t.	12.67	1
AI=5.97; p=0		
PS = 4.06; p = 0		

**, $p < 0.01$

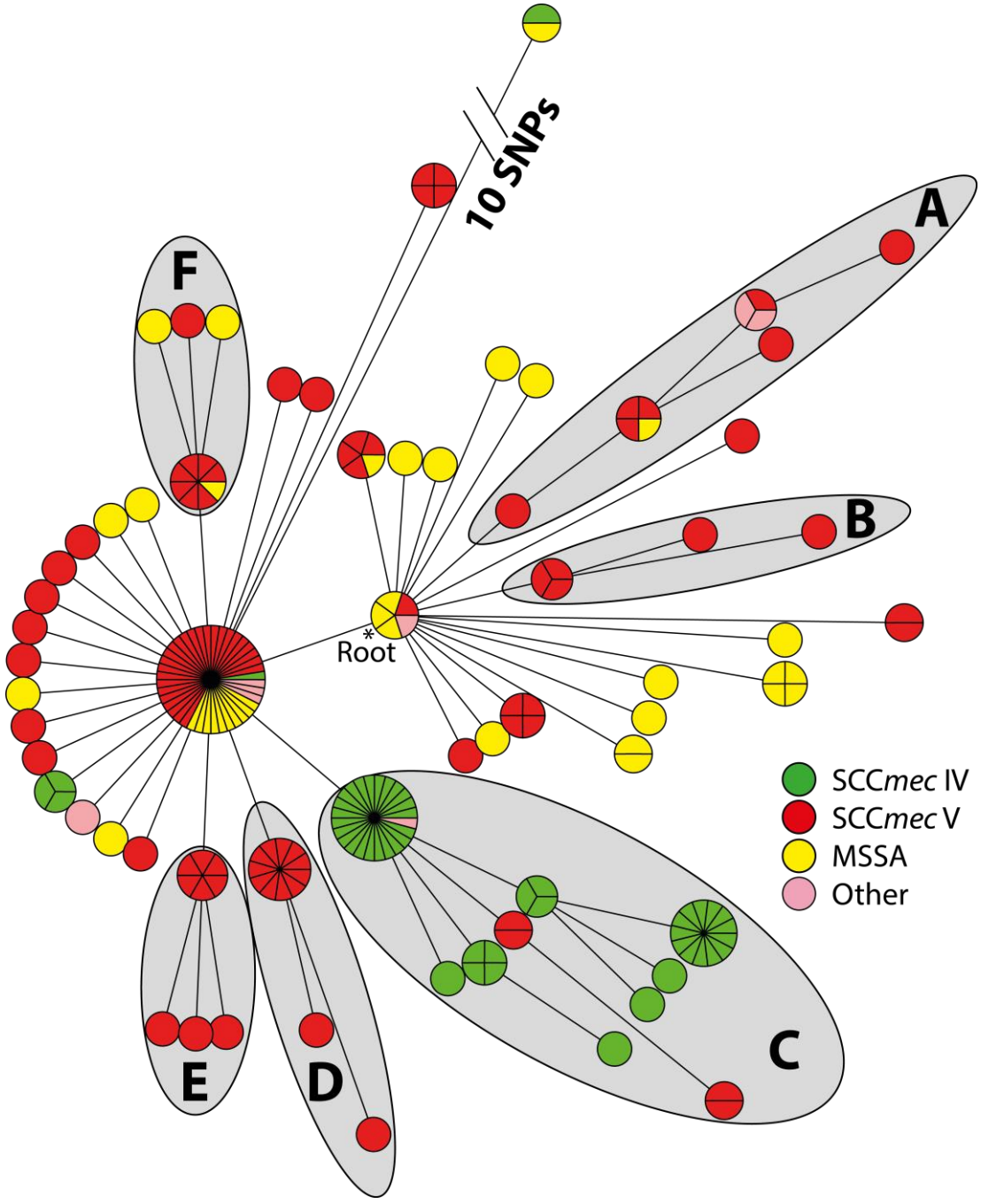


Figure 3.9. Minimum spanning tree represents the distribution of MSSA and various SCC-*mec* types among the CC398 isolates' collection.

3.3.4 Correlation of the phylogeny and host origin

The phylogenetic analysis indicated that all isolates from turkey meat (n = 4) clustered together and shared the same point mutations (Figure 3.10). These isolates were collected from Germany and were MSSA (Table A.1). In contrast, CC398 isolates recovered from human were distributed over the phylogenetic tree (Figure 3.10). Clades A, B, D and F consisted mainly of human isolates (n = 7, n = 4, n =9, n = 7; respectively). However, isolates from other host species were found in these clades. For instance, isolates from pigs (n = 2) and cat (n = 1) were located in clade A, while in clade B was one isolate collected from goat. In addition, three isolates from pigs and chicken (n = 2, n = 1; respectively) were found in clade D, whereas clade F had four isolates from pigs (Figure 3.10).

Interestingly, clade C (n = 52) consisted primarily of isolates that were collected from horses under veterinary care (n = 41) (Figure 3.10). Of note, human isolates in the same clade (n = 6; 07-00334, 07-00471, 07-01238, 07-01239, 07-01335, 07-01730) were from veterinary personnel of an equine clinic in a large Austrian veterinary hospital (Stationary Care 1) who had close contact to infected horses (Table A.1). Furthermore, 5 isolates from various hosts (dog, n = 2; bovine, n = 2; pig, n = 1) were situated in clade C (Figure 3.10); these animals were in contact with one horse (09-02432) and shared the same farm (Farm 1) (Table A.1). The CC398 isolates in clade C (n = 52) were collected from four different countries (Austria, Belgium, Germany, and the Netherlands) between 2006 and 2011 (Figure 3.7); the German isolates within this clade (n = 29) had been collected from 13 equine clinics and veterinary practitioners distributed over seven different federal states. The majority of isolates in clade C were characterised by *spa* type t011 and SCC*mec* IV (Figure 3.8, Figure 3.9, Table A.1). In total, 53 horse isolates were included in this study; four horse isolates characterized by *spa* type t034 and SCC*mec* V shared the same point mutations and were located near the root of the CC398 phylogenetic tree (Figure 3.10). In addition, estimating the TMRCA of clade C shows that this sub-lineage has emerged between horses since ≈ 2002 (95% confidence interval, 1998 to 2005).

At the same time, clade C was extremely rare among *S. aureus* CC398 isolates from human infections in Germany. Among > 6,700 isolates that had been submitted to the German National Reference Centre for Staphylococci in RKI between 2010 and

2011, there were 48 MRSA isolates from human infections that displayed *spa* type t011 (Table A.8). Among these, only four isolates carried the synonymous base substitution that defines clade C (i. e., they carried a thymidine residue at genomic position 2,533,404; SNP au309-2; Table A.7 and Table A.8), as revealed by targeted PCR and sequencing. Hence, the association of clade C with infections in horses was highly significant ($p<0.0001$; chi^2 test).

BaTS analysis indicated that CC398 isolates from turkey meat and from horses each displayed a significant association with the phylogenetic structure of the investigated CC398 isolates ($p < 0.01$; Table 3.5). In contrast, other host species including humans and pigs were more dispersed on the phylogenetic tree (Figure 3.10), not significantly different from a random distribution ($p > 0.1$; Table 3.5).

Table 3.5. BaTS analysis results show the association of isolates' host origin with the phylogeny

Host	MC value	p-value
Broiler thaw	1.2	1
Cat	1	1
Cattle	1.03	1
Chicken	1	1
Dog	1.04	1
Environment	1	1
Goat	1	1
Goose	1	1
Horse**	6.61	0.009
Human	4.58	0.19
Pig	2.37	0.47
Turkey**	4	0.009
AI=10.31; p=0		
PS = 65.36; p = 0		
**, $p < 0.01$		

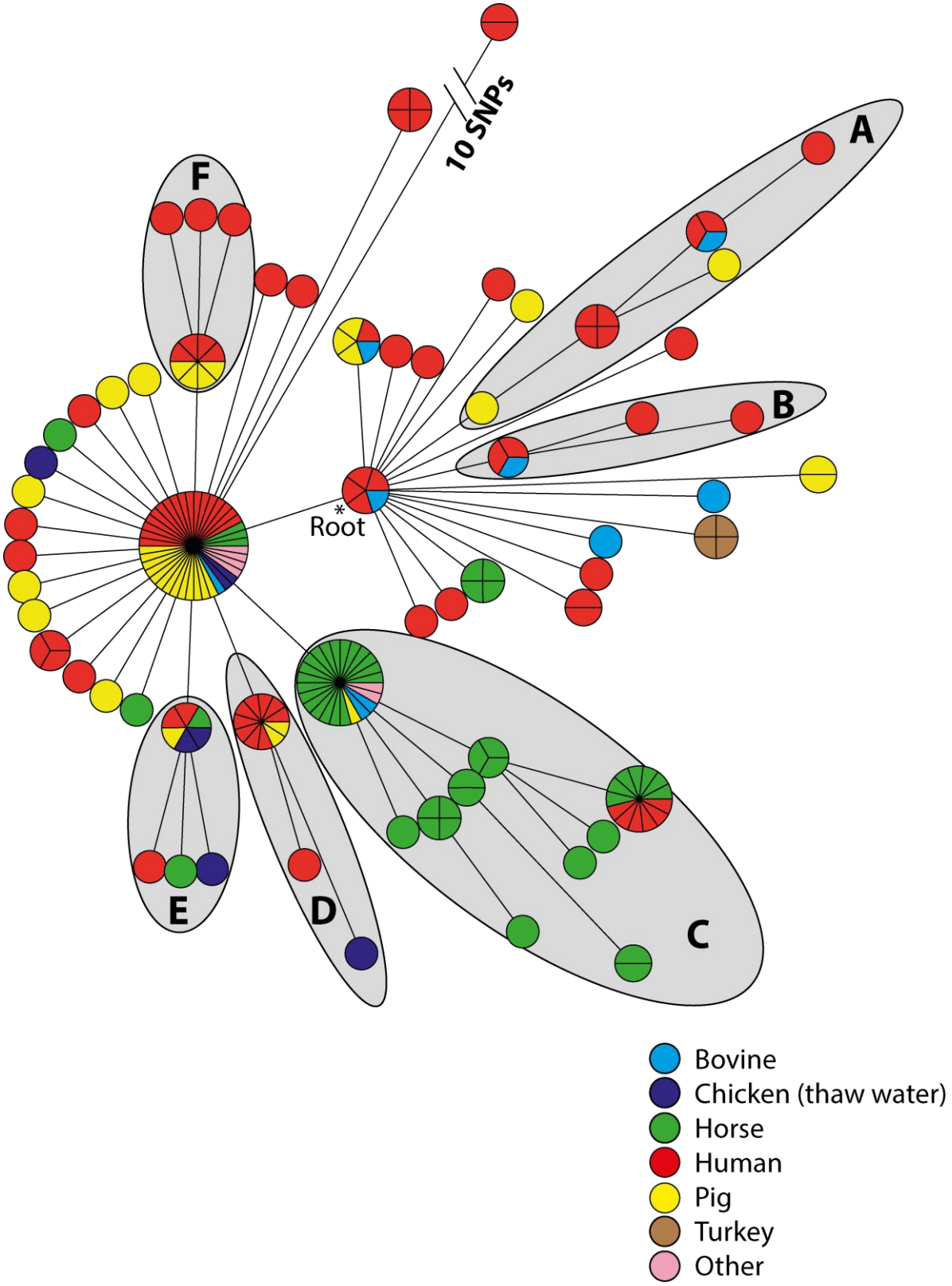


Figure 3.10. Minimum spanning tree represents the host origin of the 195 investigated CC398 isolates.

3.4 CC398 virulence factors and bacteriophages

The immune evasion cluster (IEC) genes carried on the β- converting φSa3 prophage (*sak*, *chp* and *scn*) were detected in 17 CC398 isolates (Figure 3.11.A). Eleven isolates were recovered from human (06-03005, 07-01429, 09-01311, 09-01312, 09-03229, 09-03231, 09-03234, 10-02048, 11-00078, and 11-02211), while six were of animal origin (11-00501, 11-00530, 11-01553, 11-02281, 11-02801, and 11-02802) (Table A.9). All the human isolates that carried φSa3 were MSSA, with exception of one (Table A.9). In contrast, all animal isolates that harboured the IEC genes were MRSA. Three of the animal isolates (11-02281, 11-02801, and 11-02802) were located in clade C, while the remaining three (11-00501, 11-00530, and 11-01553) were located in clade E (Figure 3.11.A).

We detected the φAvβ prophage-related sequences only in isolates from turkey meat samples (n = 4) (Table A.9). This φAvβ prophage, another member of the β-converting phage family, was detected first in avian strains belonging to CC5 [163]. In addition, the phylogenetic tree of the investigated CC398 isolates indicated that the MSSA isolates from the turkey meat samples shared the same point mutations and haplotype (Figure 3.10) and were significantly associated with the phylogeny (Table 3.5).

Panton-Valentine Leukocidin (PVL) is a cytotoxin, which is encoded by genes (lukS/F-PV) carried on bacteriophage. PVL positive *S. aureus* strains are known to cause leukocyte destruction and tissue necrosis [133]. Among the 195 investigated CC398 isolates, only seven isolates harboured the PVL genes (Figure 3.11.A). In addition, these isolates were recovered from infected human (Table A.9).

We detected bacteriophages φSa2S0385 and φSa6S0385 in 82 and 113 isolates, respectively (Figure 3.11.B). Furthermore, only 29 among the 195 investigated isolates were positive to both (φSa2S0385 and φSa6S0385) bacteriophages (Figure 3.11.B). Both bacteriophages (47 Kb) were detected first in the CC398 reference genome (strain S0385) [225], and shared common domains, which consisted of mainly genes encoding phage regulatory, tail and capsule proteins [225]. Only one virulence gene (NWMN0280) was previously identified in both phages [225]. Howev-

er, we detected these phages in clinical and commensal isolates, which were recovered from various host species.

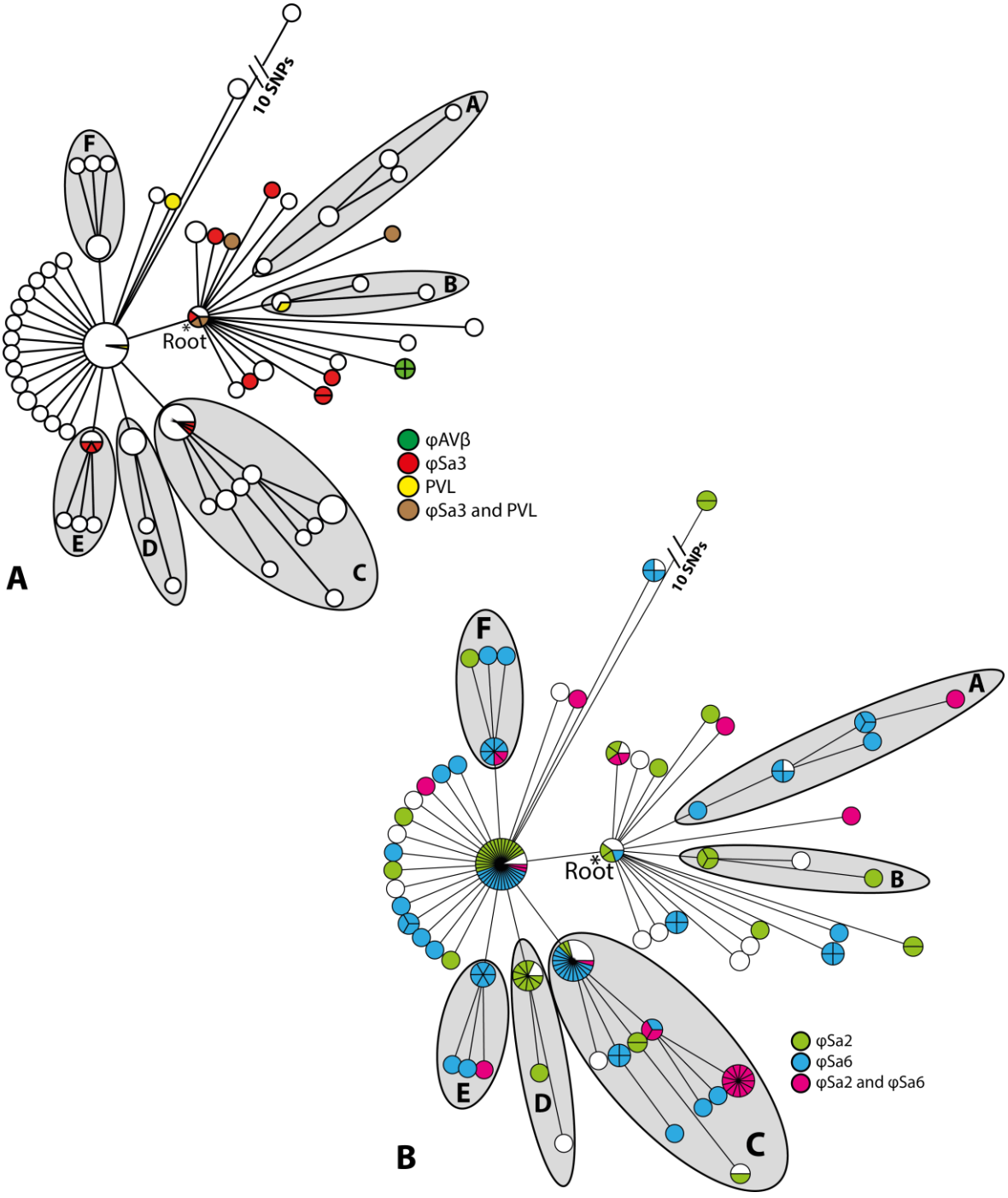


Figure 3.11. Minimum spanning tree shows the distribution of different bacteriophages that were harboured by the investigated CC398 isolates. A) φAvβ, φSa3, PVL bacteriophages detected in the 195 CC398 isolates and B) shows isolates positive for φSa2 and φSa6 bacteriophages.

3.5 Adhesion of CC398 to human and equine fibronectin

The significance of bacterial adherence followed by invasion has been previously demonstrated [228,229]. Bacteria must first adhere to the host cells before colonisation and invasion can occur. In addition, it was previously shown that the *S. aureus* fibronectin binding proteins play an important role in the invasion of mammalian cells [215]. Fibronectin (FN) is a glycoprotein and member of host extracellular matrix proteins. It is essential for different mechanisms carried out by the host cells such as tissue differentiation and wound healing. Using an *in vitro* binding assay, the adhesion ability of representative CC398 isolates to human and equine FN was studied. Six CC398 isolates were used in this assay; five isolates were recovered from equine infections, while one isolate was from pig (Table 3.6).

The FN binding assay revealed that the adherence ability of the investigated CC398 isolates (n = 6) to human and equine FN did not differ significantly ($p = 0.23$) (Figure 3.12). Furthermore, among the six investigated CC398 only one isolate recovered from a horse (10-02693) showed significantly increased adhesion ($p = 0.003$) to the equine FN compared to the human FN (Figure 3.13).

Table 3.6. CC398 isolates used in the adhesion assay.

Isolate ID	Country	Isolation Date	*Spa* type	Host	Clade
06-02016	Austria	13.04.2006	t011	Horse	C
08-00301	Germany	03.09.2007	t011	Pig	D
10-02693	Germany	05.11.2010	t034	Horse	N.A.
11-00833	Germany	12.01.2011	t6867	Horse	C
11-01925	Germany	13.05.2011	t1451	Horse	N.A.
11-02806	Germany	23.08.2011	t011	Horse	N.A.

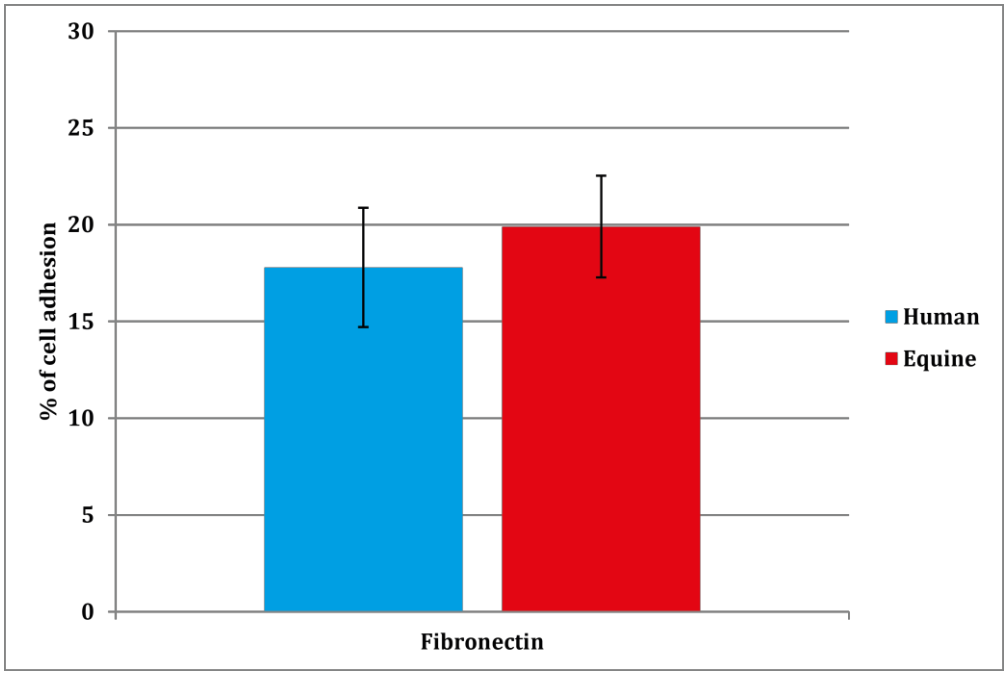


Figure 3.12. Adhesion of CC398 isolates (n = 6) to human and equine fibronectin (FN). The data represent the mean value of six independent experiments with each host's FN, while the error bars representing standard deviation.

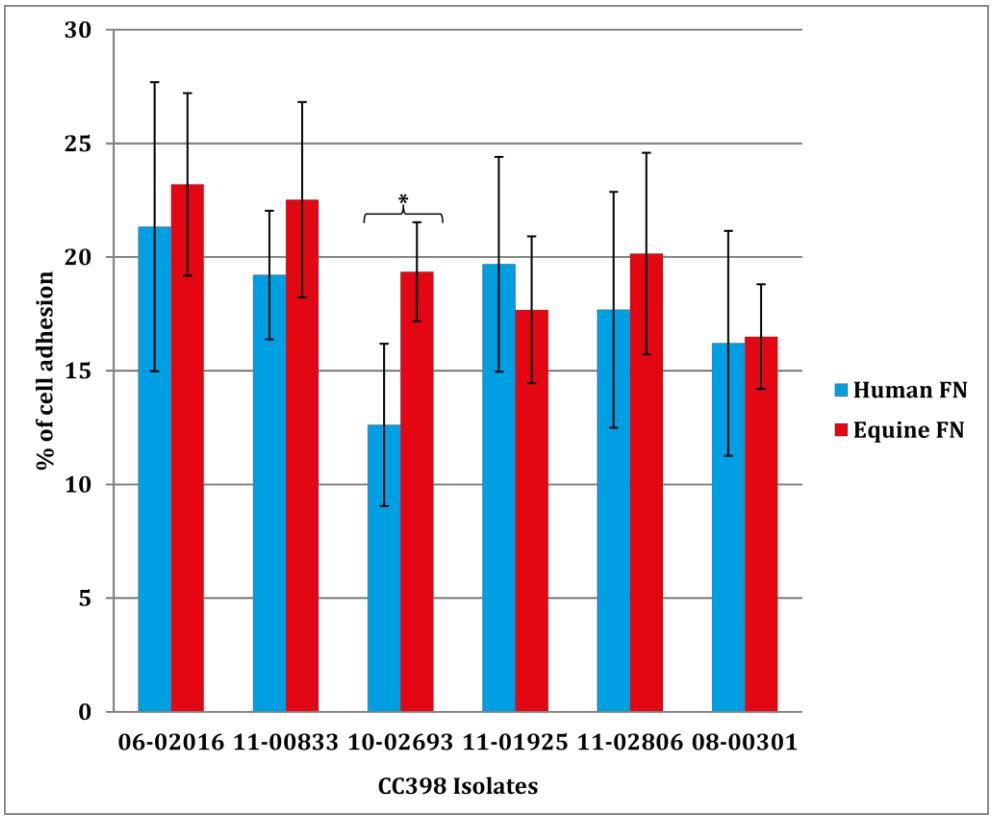


Figure 3.13. Binding of the six investigated CC398 isolates that were recovered from different hosts to human and equine fibronectin (FN). The data represent the mean values of six independent experiments with each host's FN, while the error bars representing standard deviation. Levels of significance were determined by the Mann–Whitney U test; *, $p < 0.05$.

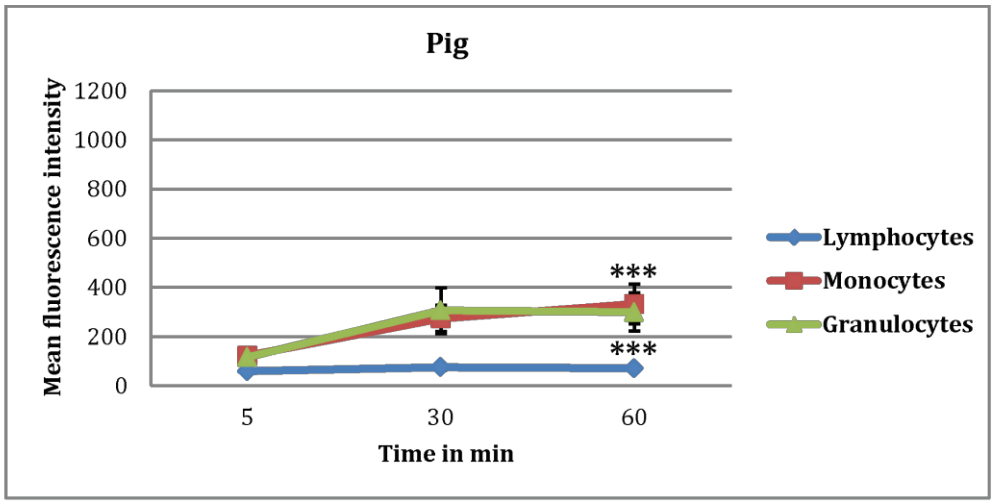
3.6 Phagocytosis of CC398 by the host's innate immune system

Phagocytosis reflects the main process for combating pathogen infections. Neutrophils or blood leucocytes are considered as the key cells of the innate immune system. Neutrophils are short-lived cells and need to be continuously generated in steady-state conditions from the bone marrow.

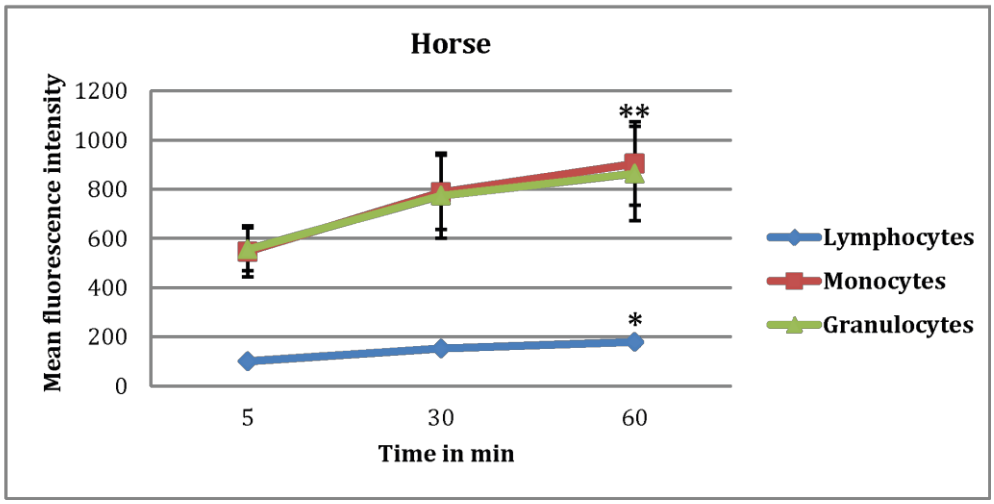
The innate immune response of various hosts (pig, horse and human) to the *S. aureus* CC398 infection was investigated. In this assay, we measured the phagocytosis of the six CC398 isolates (Table 3.6) via hosts' lymphocytes, monocytes and granulocytes at three different time points (5, 30 and 60 min) (Figure 3.14).

This analysis revealed that the phagocytosis of CC398 (n = 6) by the different host's lymphocytes, monocytes and granulocytes was significantly increased over the incubation time, with exception of human lymphocytes ($p = 0.24$) (Figure 3.14).

A



B



C

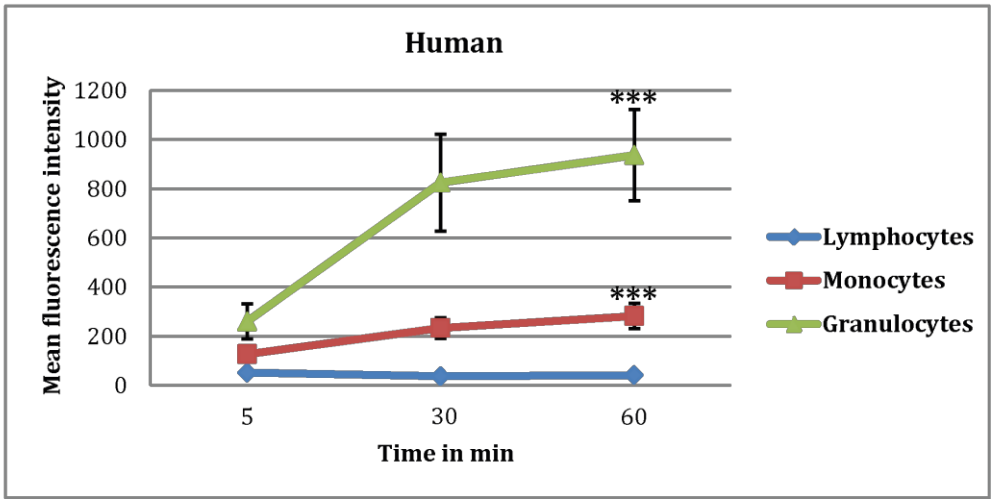


Figure 3.14. Phagocytosis of CC398 (n = 6) by various hosts' lymphocytes, monocytes and granulocytes over different time points. A) pig blood , B) horse blood, and C) human blood. The data represent the mean values of ten independent experiments with each hosts' blood, while the error bars representing standard deviation. Levels of significance were determined by the Mann–Whitney *U* test; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

In total, after 60 min of incubation, the horse lymphocytes and monocytes engulfed significantly greater number of CC398 compared to their pig and human counterparts ($p < 0.001$) (Figure 3.15). The measured phagocytosis of CC398 by pig granulocytes was significantly lower than that for both horse and human granulocytes ($p < 0.001$) (Figure 3.15). In addition, the phagocytosis of CC398 by human and horse granulocytes did not differ significantly ($P = 0.1$) (Figure 3.15).

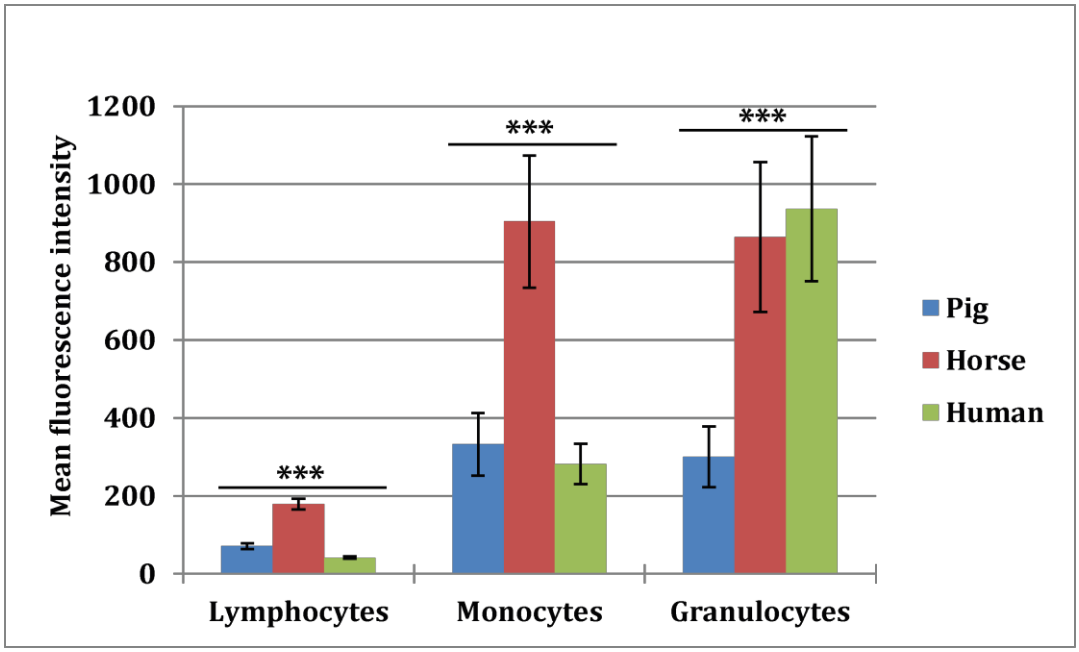


Figure 3.15. Phagocytosis of CC398 by various hosts lymphocytes, monocytes, and granulocytes after 60 min incubation time. The data represent the mean values of ten independent experiments with each hosts' blood, while the error bars representing standard deviation. Levels of significance were determined by the Mann–Whitney U test; ***, $p < 0.001$.

Figure 3.16 demonstrates the comparison of the phagocytosis of the six different CC398 isolates by host leukocytes after 60 min incubation, which revealed that monocytes and granulocytes from pig showed significantly increased phagocytosis of clade C isolates (06-02016 and 11-00833) compared to the remaining isolates ($p < 0.001$). In contrast, pig lymphocytes ingested isolates 11-02806 and 08-00301 significantly less than the remaining isolates ($p < 0.01$) (Figure 3.16.A). The phagocytosis by horse lymphocytes did not differ between isolates. Despite the fact that horse monocytes had significant reduction in the phagocytosis of the isolate recovered from pig (08-00301) compared with other isolates ($p < 0.001$). Granulocytes from horse ingested significantly higher amount of isolates 06-02016, 11-00833 and 10-02693 than the remained three isolates ($p < 0.01$) (Figure 3.16.C). Of note, phagocytosis by human lymphocytes, monocytes and granulocytes was not isolate dependent (Figure 3.16).

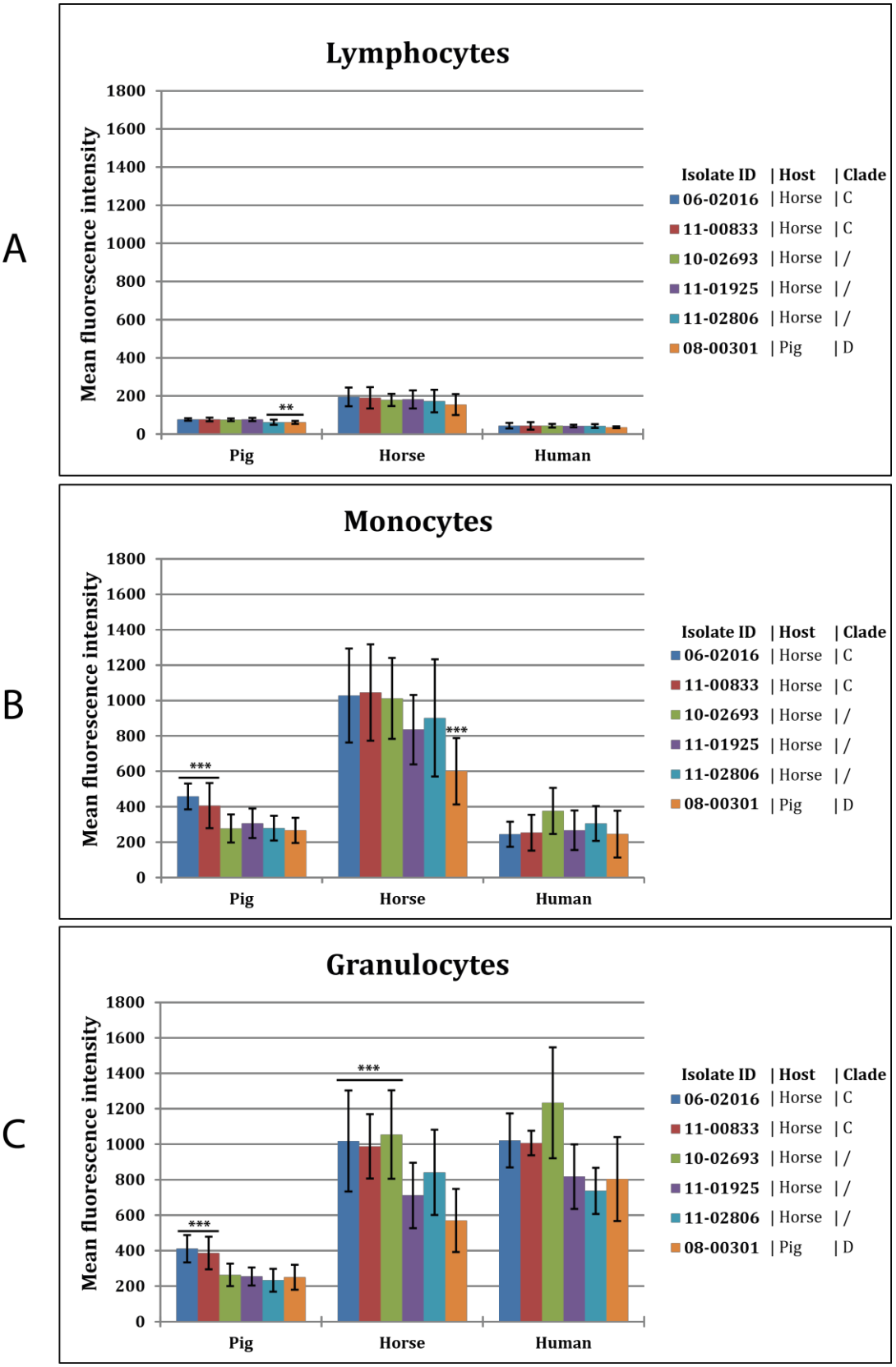


Figure 3.16. Phagocytosis of the six different CC398 isolates by lymphocytes (A), monocytes (B) and granulocytes (C) of pig, horse and human, after 60 min incubation time. **, $p < 0.01$; ***, $p < 0.001$.

3.6.1 Immune evasion cluster and phagocytosis of CC398

The *S. aureus* immune evasion cluster (IEC), located on β-haemolysin-converting bacteriophages, encodes the immune-modulating proteins chemotaxis inhibitory protein (CHIP), staphylococcal complement inhibitor (SCIN), staphylococcal enterotoxin A (SEA) and staphylokinase (SAK). This IEC mediates the *S. aureus* invasion by escaping the host innate immunity. Previous study demonstrated that CC398 originated in human where it acquired the prophage ΦSa3, which carried genes encoding the IEC (Price et al, 2012).

To study influence of the IEC genes on the host innate immunity, two groups of CC398 isolates (with and without the IEC genes) were used to measure their phagocytosis by human leukocytes (lymphocytes, monocytes and granulocytes). These CC398 were mainly recovered from infection sites from both animal and human (Table 3.7).

Table 3.7. CC398 isolates used for investigating the influence of acquisition the IEC on phagocytosis.

Isolate	Country	Isolation date	*Spa* type	SCC*mec*	Host	Characteristic	Clade	φSa3
11-02281	Germany	30/06/11	t779	II	Horse	Clinical isolate	C	Positive
11-02801	Germany	14/08/11	t011	IV	Horse	Clinical isolate	C	Positive
11-02802	Germany	14/08/11	t011	IV	Horse	Clinical isolate	C	Positive
06-03005	Germany	01/12/06	t571	MSSA	Human	Wound infection	Not defined	Positive
11-00501	Germany	10/02/11	t034	V	Thaw water	No information	E	Positive
11-00530	Germany	14/02/11	t034	V	Thaw water	No information	E	Positive
11-01553	Germany	26/04/11	t034	V	Horse	Clinical isolate	E	Positive
11-02407	Germany	03/08/11	t1451	MSSA	Human	Wound infection	Not defined	Positive
09-03591	Germany	03/12/09	t011	MRSA	Human	Clinical isolate	Not defined	Positive
09-01897	Germany	11/05/09	t034	MRSA	Human	Clinical isolate	Not defined	Positive
10-01386	Germany	18/06/10	t034	MRSA	Human	Clinical isolate	Not defined	Positive
07-01429	Germany	01/06/07	t571	MSSA	Human	Wound infection	Not defined	Positive
11-02804	Germany	19/08/11	t011	IV	Horse	Clinical isolate	C	Negative
11-02560	Germany	18/07/11	t011	IV	Horse	Clinical isolate	C	Negative
11-01189	Germany	04/04/11	t011	IV	Horse	Clinical isolate	C	Negative
07-01494	Germany	01/06/07	t034	V	Human	Wound infection	A	Negative
11-01119	Germany	21/03/11	t034	V	Horse	Clinical isolate	E	Negative
09-00709	Germany	01/06/09	t034	V	Human	Commensal	E	Negative
07-01653	Germany	01/06/07	t034	V	Human	Wound infection	E	Negative
09-00393	Germany	01/06/09	t034	V	Human	Commensal	Not defined	Negative
07-00415	Germany	01/06/07	t034	MSSA	Human	No information	Not defined	Negative
09-00444	Germany	01/06/09	t011	V	Human	Commensal	E	Negative
07-02239	Germany	01/06/07	t108	V	Human	Wound infection	B	Negative
08-02710	Germany	06/10/08	t034	MSSA	Human	No information	A	Negative

Acquisition of the IEC by CC398 isolates did not influence the phagocytosis by human lymphocytes and monocytes after 60 min incubation time (Figure 3.17). In contrast, human granulocytes showed significant reduction in the phagocytosis of CC398 isolates with IEC after 30 and 60 min (p = 0.002, P = 0.001; respectively) compared to IEC negative CC398 isolates (Figure 3.18).

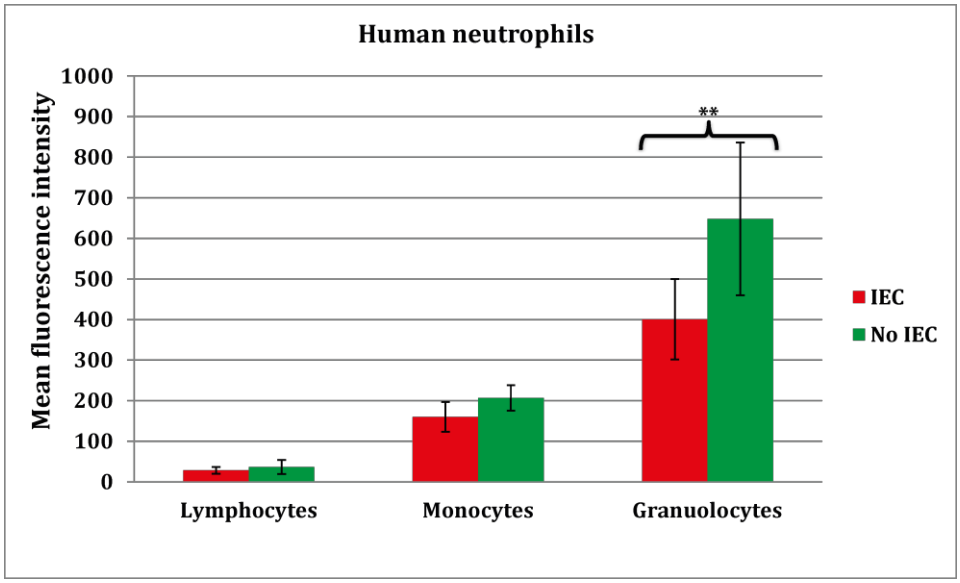


Figure 3.17. Human neutrophils after 60 min and the phagocytosis of CC398 with and without immune evasion cluster (IEC). **, p < 0.01.

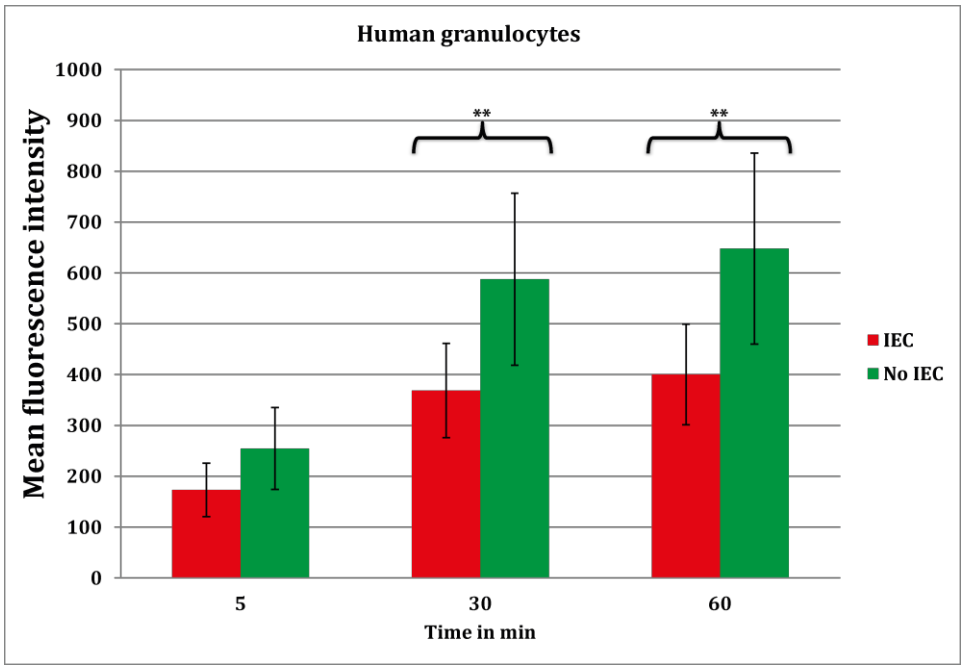


Figure 3.18. Phagocytosis of CC398 with and without immune evasion cluster (IEC) by human granulocytes. **, p < 0.01.

3.7 Whole genome sequence analysis

The evolution and epidemiology of CC398 was elucidated using a dHPLC-based mutation discovery method. This dHPLC approach covered 1.4% of the CC398 genome, and delivered higher discriminatory power compared to the standard *spa* and MLST typing methods. However, to assess the molecular genetic basis that lead to successful spread of clade C between horses with improved discriminatory resolution, we sequenced the genomes of six representative CC398 isolates (Table 3.8). These isolates were distributed over the CC398 phylogenetic tree, and their genomes were 454 pyrosequenced using Roche genome sequencer Titanium. In addition, raw Illumina sequence data of two CC398 isolates from horse (IMT25053, wound infection, t011, SCC*mec* V; IMT26596, wound infection, t6867, SCC*mec* IV) were obtained from the Institute of Microbiology and Epizootics at the Free University Berlin as a part of the MedVet Staph project's cooperation (Table 3.8). All eight genome sequences were *de novo* assembled; the six 454 pyrosequenced genomes were assembled using Newbler software; while the two Illumina sequenced genomes were assembled using Velvet software.

Table 3.8. CC398 isolates used for whole genome sequencing.

Isolate ID	Sequencing technology	Number of reads	Average read length (bp)	Total bases (bp)	Coverage	Number of contigs	Number of bases in contigs (bp)
06-02016	454/ Roche	340109	608.63	207,001,138	69	132	2,828,647
08-00301	454/ Roche	400641	611.49	244,988,774	82	74	2,806,552
10-02693	454/ Roche	214392	598.18	128,244,254	43	51	2,740,366
11-00833	454/ Roche	202229	591.43	119,605,158	40	58	2,764,070
11-01925	454/ Roche	184929	592.26	109,525,161	37	40	2,769,179
11-02806	454/ Roche	154281	602.73	92,990,274	31	50	2,848,200
IMT25053	Illumina	3734559	101	377,190,459	131	50	2,756,033
IMT26596	Illumina	3887464	101	392,633,864	137	53	2,802,118

3.7.1 Comparative genomics

The eight sequenced genomes (06-02016, 08-00301, 10-02693, 11-00833, 11-01925, 11-02806, IMT25053, and IMT26596) (Table 3.8) were compared to the sequenced LA-MRSA CC398 reference genome (strain S0385; accession no. AM990992), which characterized by *spa* type t011, SCC*mec* V, and recovered from human infection in the Netherlands. In addition, we included in this comparison three previously published CC398 genome sequences [192,230], which were isolated from human infections. Among these three complete genomes, two were MSSA from USA with *spa* type t571 (ST398NM01, accession number CP003045; ST398NM02, accession number AIDT00000000 [192]), while the third genome was for a Canadian representative LA-MRSA isolate with *spa* type t034 (08BA02176, accession number CP003808 [230]) (Figure 3.19, Table 3.9).

The genomic comparisons represented in Figure 3.19 revealed that the investigated CC398 genomes and the reference genomes (strain S0385) were diverse in terms of mobile genetic elements such as prophages, transposons and SaPIs. Similar to the reference (strain S0385) and other CC398 complete genomes, the eight investigated genomes lacked various virulence factors present in other epidemic MRSA strain types, such as enterotoxin and exfoliative toxin genes [225,230,231]. Furthermore, the genomic islands νSaα and νSaβ were identified in all genomes (Table 3.9). These islands were detected in the reference genome (strain S0385) and carried type I restriction-modification (R-M) system, which is essential for preventing horizontal gene transfer into the bacterial genome [225]. Only isolates 11-00833 and IMT26596 carried stretches of the integrative conjugative elements (ICESa1A, ICESa1B and ICESa2) (Figure 3.19), which were detected previously in the reference strain (S0385) [225].

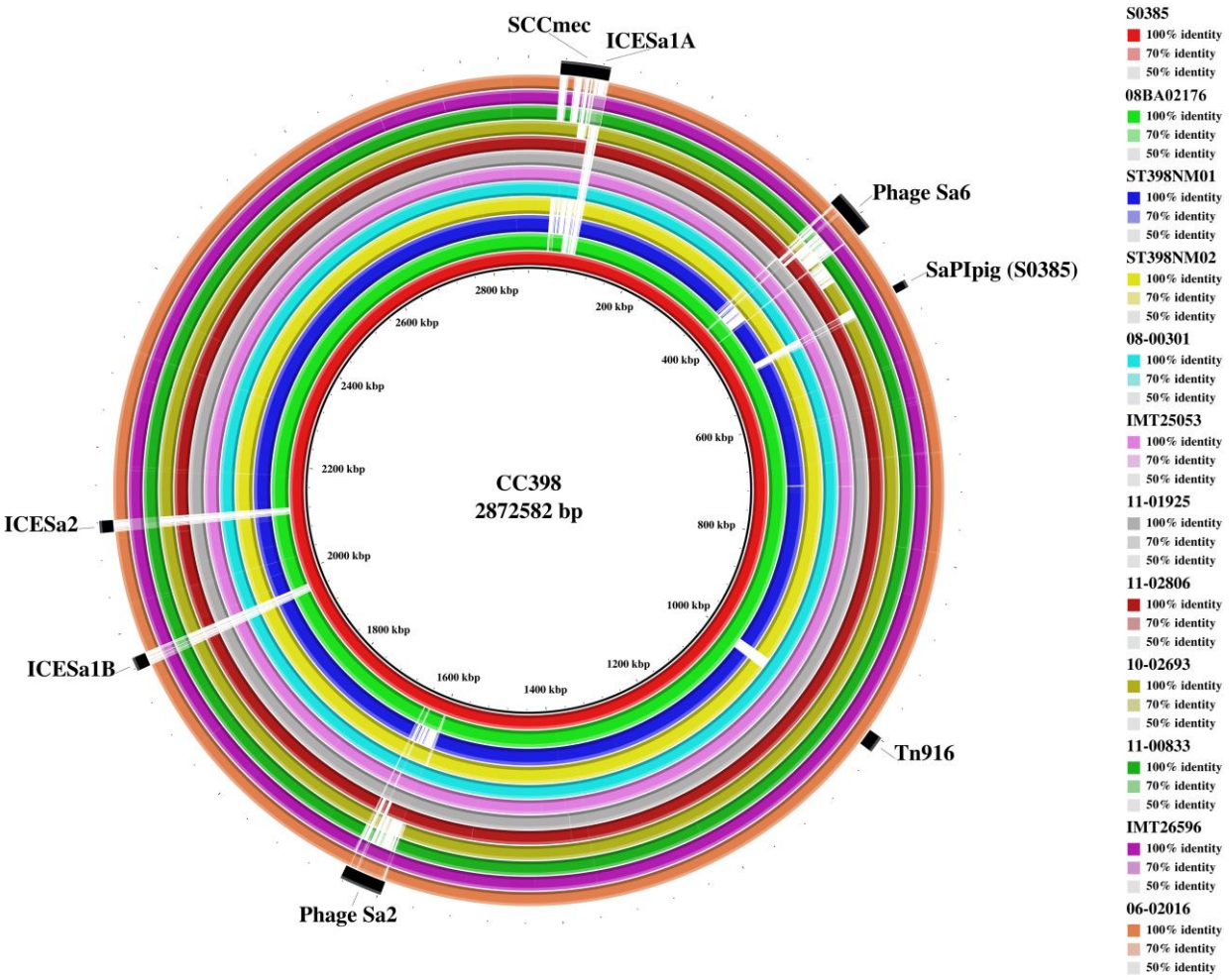


Figure 3.19. Comparison of different CC398 genomes. Each circle represents different genome, from inside: LA-MRSA reference genome (S0385, inner ring), while the outer ring represents isolate 06-02016 genome.

Table 3.9. Genomic content of the investigated CC398 isolates.

				Isolate (clade)											
Mobile genetic elements [a]	**Locus tag**	**Position in the reference genome (strain S0385, accession no. AM990992)**	**Size in bp**	**S0385 (D)**	**08BA02176**	**ST398NM01**	**ST398NM02**	**08-00301 (D)**	**IMT25053 (N.A)**	**11-01925 (N.A)**	**11-02806 (N.A)**	**10-02693 (N.A)**	**11-00833 (C)**	**IMT26596 (C)**	**06-02016 (C)**
SCC*mec*	SAPIG0028- SAPIG0085	34531- 88218	53,687	V	V	-	-	V	V	V	V	V	IV	IV	IV
SaPI Pig (S0385)	SAPIG0469-SAPIG0483	484555- 496868	12,313	X	X	-	-	-	-	-	-	-	X	X	X
SaPIbov4-Like			13,606	-	-	-	-	-	X	X	X	-	-	-	-
νSaα (R-M & hsdS genes)	SAPIG0499-SAPIG0500	511859- 514586	2,727	X	X	X	X	X	X	X	X	X	X	X	X
Type II R-M	SAPIG2545-SAPIG2546	2625780- 2627998	2,218	X	X	X	X	X	X	X	X	X	X	X	X
ICESa1A	SAPIG0070- SAPIG0084	75635- 87783	12,148	X	-	-	-	-	-	-	-	-	X	X	-
IECSa1B	SAPIG1847- SAPIG1866	1956685- 1971358	14,673	X	-	-	-	-	-	-	-	-	-	-	-
ICESa2	SAPIG2030-SAPIG2046	2108820- 2121908	13,088	X	-	-	-	-	-	-	-	-	X	X	-
Transposase	SAPIG1293	1356636-1357583	947	X	X	X	-	-	-	-	-	-	-	-	-
Tn916-like transposon	SAPIG0953-SAPIG0969	997945- 1016147	18,202	X	X	-	-	X	X	X	X	X	X	X	X
Tn554 like transposon	SAPIG2023-SAPIG2025	2102165- 2105717	3,552	X	X	X	X	X	X	X	X	X	X	X	X
Transposon Tn559-like	Inserted in SAPIG1715 (DNA repair protein RadC)		4,214	-	-	-	-	-	-	-	-	X	X	X	X
Tn7_like transposon	SAPIG2202- SAPIG2208	2276988- 2283585	6,597	X	X	X	X	X	X	X	X	X	X	X	X
Sa2S0385 Prophage	SAPIG1489-SAPIG1554	1597005- 1643185	46,180	X	-	-	X	X	X	X	X	-	-	-	X
Sa6S0385 Prophage	SAPIG0334-SAPIG0399	370475- 417309	46,834	X	X	-	-	-	-	X		X		X	X
Phage1			≈44,000	-	-	-	-	-	-	-	-	-	X	X	-
Phage2			≈47,000	-	-	-	-	-	-	-	X	-	-	-	-

[a], the presence of a mobile genetic element is represented by (X), while its absence is represented by (-)

3.7.1.1 Surface proteins

S. aureus adheres to components of the extracellular matrix (ECM) of the host to facilitate colonisation and infection. This adherence is mediated by so-called microbial surface proteins, which are anchored to the *S. aureus* cell wall. A previous study, based on whole genome sequencing, reported variation in genes encoding surface proteins in the LA-MRSA CC398 reference genome (strain S0385) compared with the human MSSA CC398 strains (ST398NM01 and ST398NM02) [192]. For instance, a number of surface proteins in the LA-MRSA CC398 (strain S0385) harboured premature stop codons that lead to generation of protein truncation [192]. In contrast, the human MSSA CC398 isolates had no truncations in these surface proteins "dubbed wild type" [192].

In order to assess the genetic variation of the surface proteins from horse, human and LA-CC398 isolates, we compared the gene sequences of 14 surface proteins that are listed in Table 3.10. In contrast to the human and the horse CC398 isolates, the reference strain S0385 lacked the *sdrE* gene (Table 3.10). Variations ranged between point mutation to insertions or deletions (Indels) were detected among 12 surface proteins gene sequences (Table 3.10).

Interestingly, *clfA* gene from clades C isolates had an insertion of 18 bp compared with its counterpart from the remaining isolates (Table 3.10). In contrast to the reference strain (S0385), there was no truncation of *clfB* gene in the eight investigated isolates, but it harboured Indels in the variable repeat region ranged between 18 to 42 bp (Table 3.10). Among the horse CC398 isolates, only isolate 10-02693 harboured the genes encoding *fnbB* and *sdrC* that were similar to the human CC398 (ST398NM01) (Table 3.10).

Table 3.10. Variation of CC398 isolates surface proteins

		Isolate (clade)											
Surface protein	**Gene***	**ST398-NM01 (Human-CC398)**	**ST398-NM02 (Human CC398)**	**S0385 (LA-CC398)**	**08BA02176 (Human CC398)**	**08-00301 (D)**	**IMT25053 (N.A)**	**11-01925 (N.A)**	**11-02806 (N.A)**	**10-02693 (N.A)**	**11-00833 (C)**	**IMT26596 (C)**	**06-02016 (C)**
Clumping factor A	***clfA***	WT/6 SNPs	WT/6 SNPs	Truncated/ 6 SNPs	WT/6 SNPs	WT/Δ 24 bp /Δ 139/6 SNPs bp	WT/Δ 24 bp /Δ 139/6 SNPs bp	WT/Δ 24 bp /Δ 139/6 SNPs bp	WT/Δ 24 bp /Δ 139/6 SNPs bp	WT/Δ 24/Δ 139/4 SNPs	WT/Δ 18/6 SNPs	WT/Δ 24/Δ 139/6 SNPs	WT/Δ 24/Δ 139/Δ 18/6 SNPs
Clumping factor B	***clfB***	WT	Δ 456 bp	Truncated	WT	WT/Δ 18 bp	WT/Δ 18 bp	WT/Δ 18 bp	WT/Δ 42/Δ 19 bp	WT/Δ 18 bp	WT/Δ 18 bp	WT/Δ 18 bp	WT/Δ 18 bp
Collagen	***cna***	ΔB domain	ΔB domain	WT	Δ 561	Δ 561	Δ 561	Δ 561	Δ 561	Δ 561	Δ 561	Δ 561	Δ 561
Coagulase	***coa***	WT	WT	Δ81 bp	Δ243 bp	WT	WT	WT	WT	WT	WT	WT	WT
Elastin-binding protein of S. aureus	***ebps***	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Extracellular complement-binding protein	***ecb***	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Fibronectin binding protein A	***fnbA***	WT	Δ42 bp	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
Fibronectin binding protein B	***fnbB***	WT	WT	Truncated	Truncated	Truncated	Truncated	Truncated	Truncated	WT	Truncated	Truncated	Truncated
Iron-regulated surface determinants B	***isdB***	WT	WT	1 SNP/Δ9 bp	WT	1 SNP/Δ9 bp	1 SNP/Δ9 bp	1 SNP/Δ9 bp	1 SNP/Δ9 bp	WT/1 SNP	1 SNP/Δ9 bp	1 SNP/Δ9 bp	1 SNP/Δ9 bp
Iron-regulated surface determinants C	***isdC***	WT	WT	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP	WT/ 1 SNP
Serine aspartate repeat protein C	***sdrC***	WT	WT	Δ174 bp	WT	Δ174 bp	Δ174 bp	Δ174 bp	Δ174 bp	WT	Δ174 bp	Δ174 bp	Δ174 bp
Serine aspartate repeat protein D	***sdrD***	Δ54 bp	WT	3 SNPs	WT	WT	WT	WT	WT	WT	WT	WT	WT
Serine aspartate repeat protein E	***sdrE***	WT/Δ 54	Δ138 bp	Absent	WT	WT	WT	WT	WT	WT/Δ 18	WT	WT	WT/Δ 18
Serine aspartate repeat protein H	***sdrH***	Δ39 bp	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT

* WT, wild type

3.7.1.2 Mobile genetic elements

Previous studies have shown that certain mobile genetic elements contribute to the successful spread of CC398 between human and livestock [190,192]; therefore, we determine the presence of transposons, pathogenicity islands, and prophages in the investigated CC398 genomes.

3.7.1.2.1 Transposons

The entire investigated genomes harboured the transposons Tn*916*, Tn*552* and Tn*7*-like (Table 3.9), which were previously identified in the reference LA-MRSA CC398 genome (strain S0385) [225]. The Tn*916* was found to carry the tetracycline resistance element *tet*(M), while Tn*552* carries regulator components that involve in tetracycline and β-lactam resistance [225].

Among the investigated isolates, only isolate 10-02693 and the entire clade C isolates (11-00833, IMT26596 and 06-02016) harboured a transposon similar to Tn*559* in their chromosomes (Table 3.9). This Tn*559* was found to carry gene encoding trimethoprim resistance (*dfrK*) [232].

3.7.1.2.2 Pathogenicity islands

Similar to the LA-CC398 reference strain (S0385) and 08BA02176 genomes, isolates from clade C (06-02016, 11-00833 and IMT26596) harboured the SaPI (SaPIpig S0385) (Figure 3.19, Table 3.9). This SaPIpig carry genes encoding two putative extracellular proteins homologous to the staphylococcal complement inhibitor (*scn*) and von Willebrand factor-binding protein (*vwb*), which were previously reported for their association with host specificity in ruminant [52,225,230]. In contrast, isolates 08-00301 (*spa* type t011, from pig) and 10-02693 (*spa* type t034, from horse) lacked any SaPI (Figure 3.19, Table 3.9).

Interestingly, the horse isolates 11-01925, 11-02806 and IMT25053 harboured a novel SaPI ≈ 13 kb in length (dubbed SaPIbov4-like), which showed a high level of sequence similarity to the SaPIbov4 (accession number HM211303) rather than to SaPIpig (S0385) (Figure 3.20). This SaPIbov4-like carried a set of typical SaPI genes such as integrase (*int*), *vwb* and *scn*, which were similar to their counterparts from SaPIbov4 by 98%, 94% and 94%; respectively (Figure 3.21). In contrast to SaPIbov4, SaPIbov4-like carried three additional genes encoding hypothetical proteins (Figure 3.22).

Although isolates 11-01925, 11-02806 and IMT25053 harboured the SaPIbov4-like, however, genetic variation was observed between these isolates concerning the *vwb* gene (Figure 3.23). In SaPIbov4-like, the *vwb* gene was 1,491 bp in length and encoded 496 amino acid residues. The *vwb* sequences from isolates 11-02806, IMT25053 and 11-01925 were found to be completely identical in all but one nucleotide, leading to the substitution of an asparagine by a lysine (Figure 3.23).

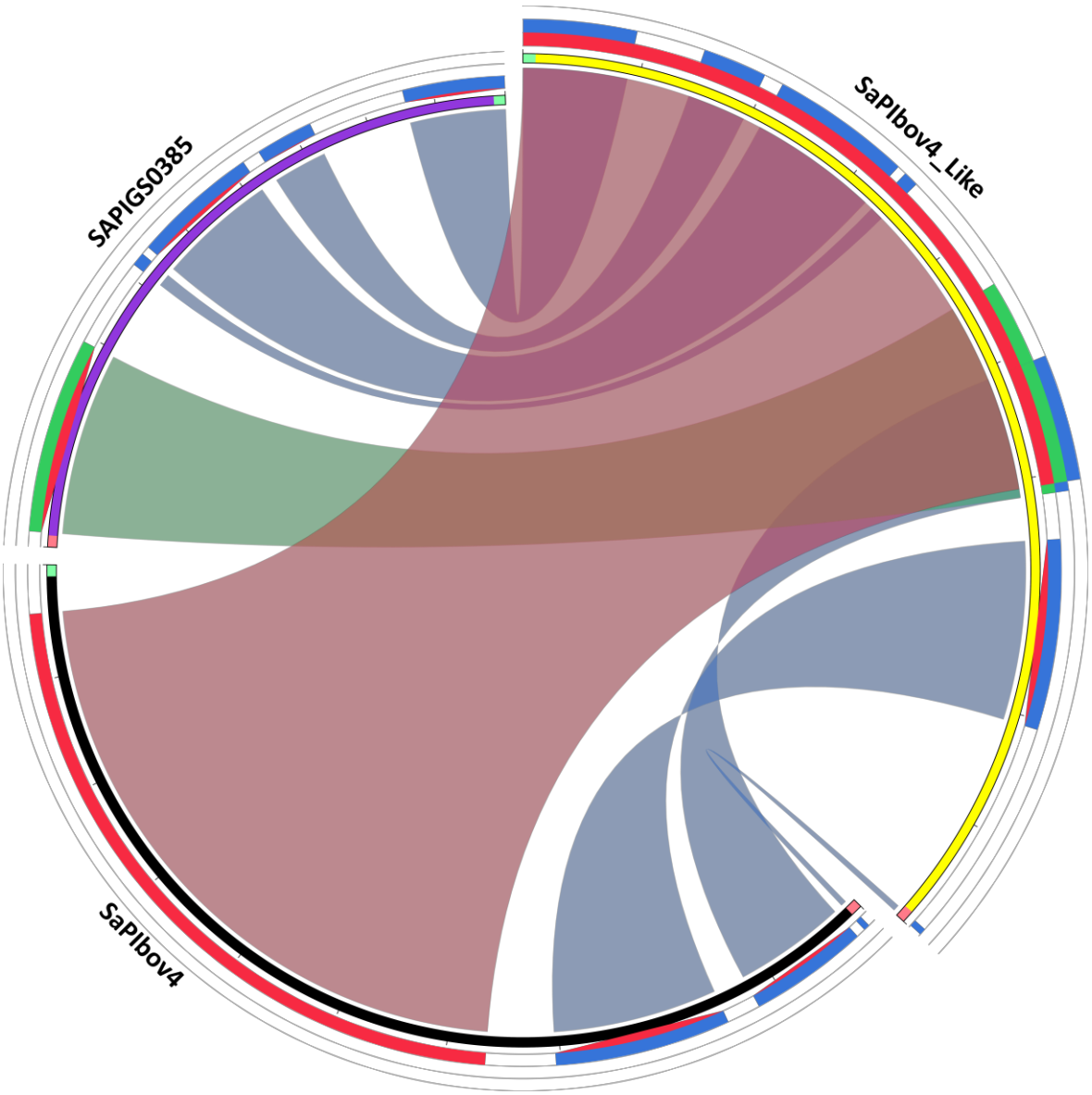


Figure 3.20. Similarity of the novel SaPI (SaPIbov4-like) with SaPIGS0385 and SaPIbov4 (accession number AM990992 and HM211303; respectively). The different degrees of DNA sequences similiarities 80%, 50%, 20% are represented by the colours red, blue and green; respectively.

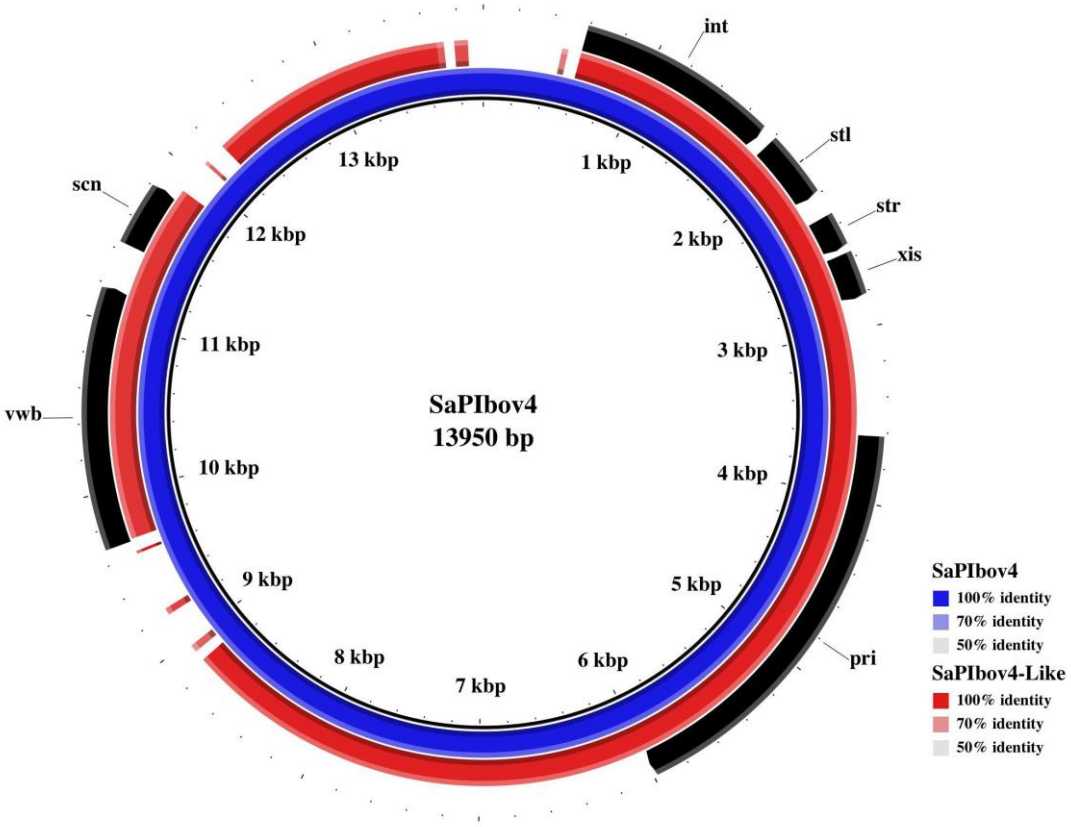


Figure 3.21. Comparison of the shared genes between SaPIbov4 (blue) and SaPIbov4-like (red).

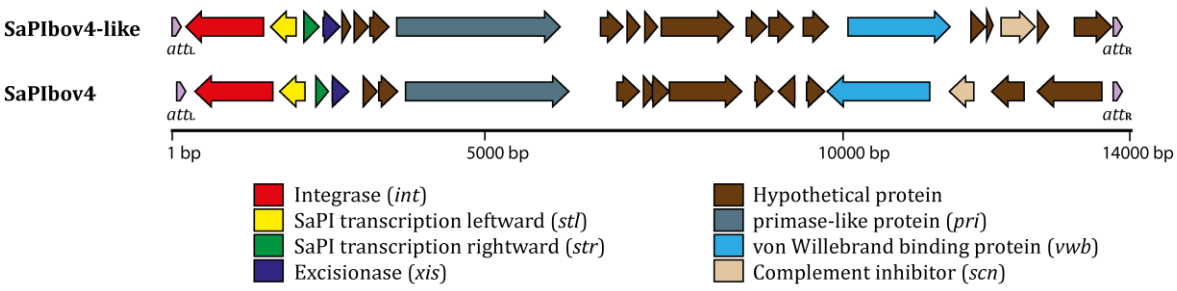


Figure 3.22. Comparison of SaPIbov4-like and SaPIbov4 genetic contents.

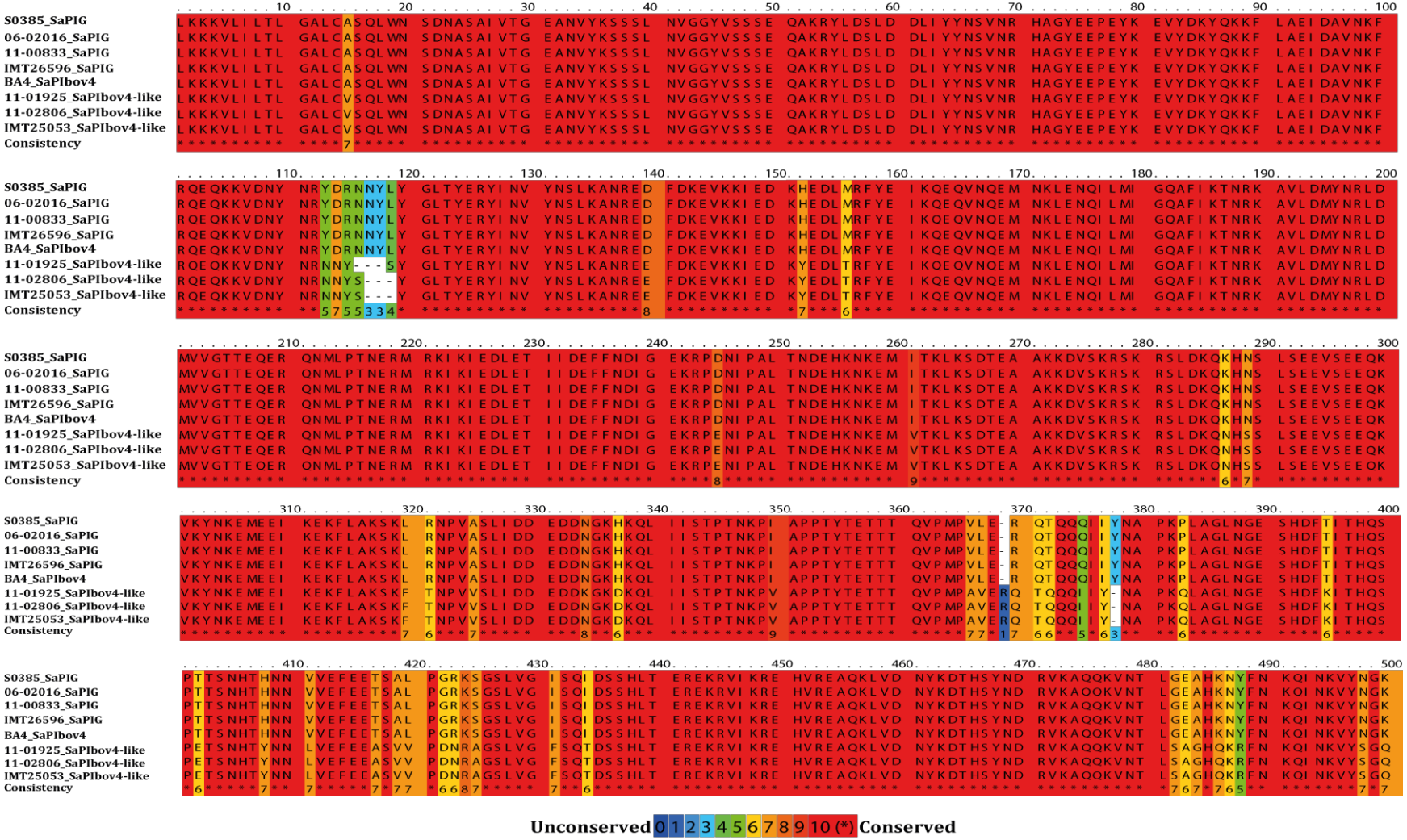


Figure 3.23. Line-up of the von Willebrand factor-binding protein (*vwb*) sequences (residues 1-500) obtained from SaPIbov4, SaPIG (strain 385) and SaPIbov4-like, which were harboured by different CC398 isolates. This figure is adapted from line-up generated by PRALINE tool. The different colours represent the relative sequence conservation at each position, and the conservation scoring was performed by PRALINE. The scoring scheme works from 0 for the least conserved alignment position, up to 10 (*) for the most conserved alignment position.

3.7.1.2.3 Prophages

Among the investigated isolates, 06-02016, 08-00301, 11-01925, 11-02806 and IMT25053 carried prophage φSa2S0385 (Figure 3.19), while the genes present in prophage φSa6S0385 were detected only in three isolates (06-02016, 10-02693 and IMT26596) (Figure 3.19) (Table A.9). These φSa2S0385 and φSa6S0385 prophages were previously described for the LA-CC398 reference strain (S0385) [225].

Three horse CC398 genomes (11-00833, IMT26596 and 11-02806) contained stretches of ≈44 and ≈47 kb of novel prophages DNA sequences (Phage1 and Phage2, respectively). Both prophages (Phage1 and Phage2) had a mosaic structure and consisted mainly of genes encoding phage regulatory proteins (head, tail and capsule proteins) (Figure 3.24), which were similar to previously detected prophages sequences from other *S. aureus* genomes (Figure 3.25). The two phages differed at the terminal end, which included the integrase gene (Figure 3.25). The integrase gene of Phage1 found in isolates 11-00833 and IMT26596 was highly similar (99%) to the integrase gene (SA2981_0802) from previously published *S. aureus* genome 04-02981 (accession no. CP001844) [106]. Similarly, the integrase gene of Phage2 in isolates 11-02806, was 98% similar to the integrase gene in genomes ED133 (SAV_1817) and LGA251 (SARLGA251_17130) (accession no. CP001996 and FR821779, respectively) [33,233]. However, no virulence determinants were detected for both phages (Phage1 and Phage2).

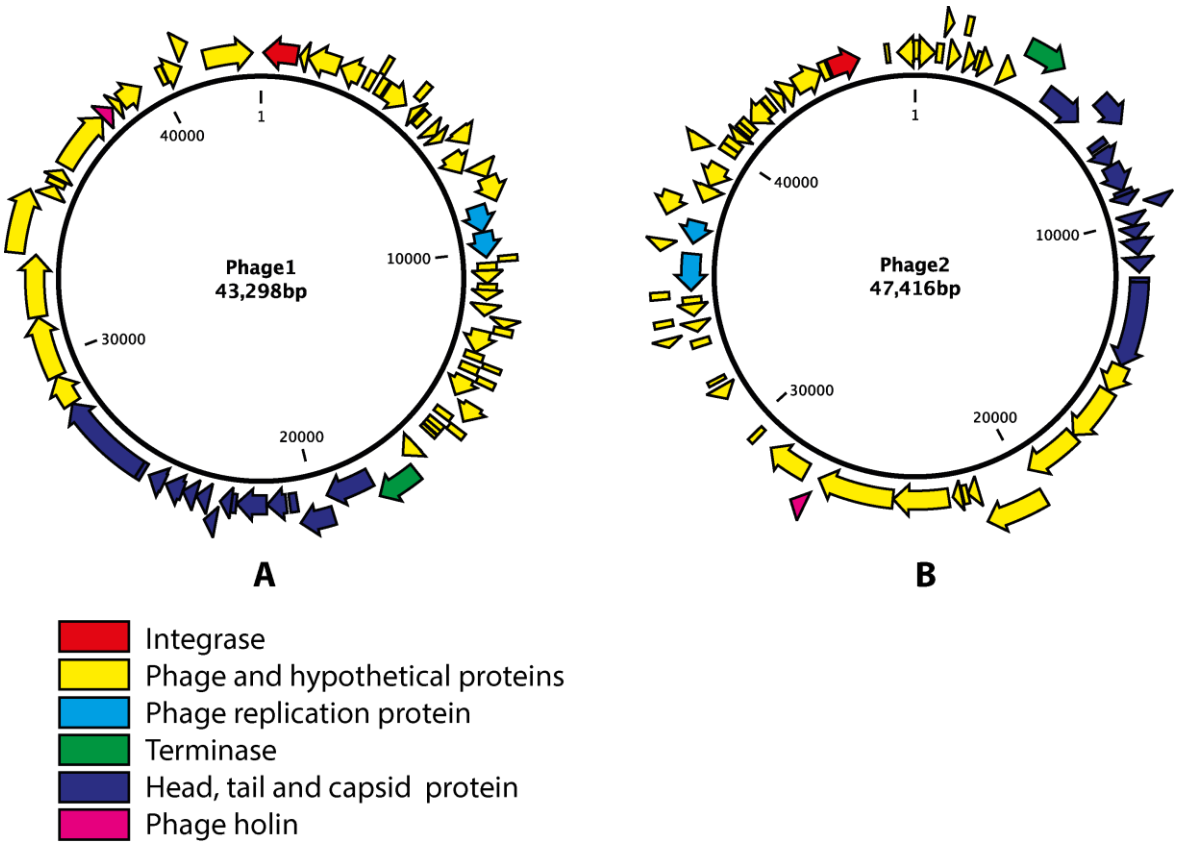


Figure 3.24. Structure of phage1 and phage2

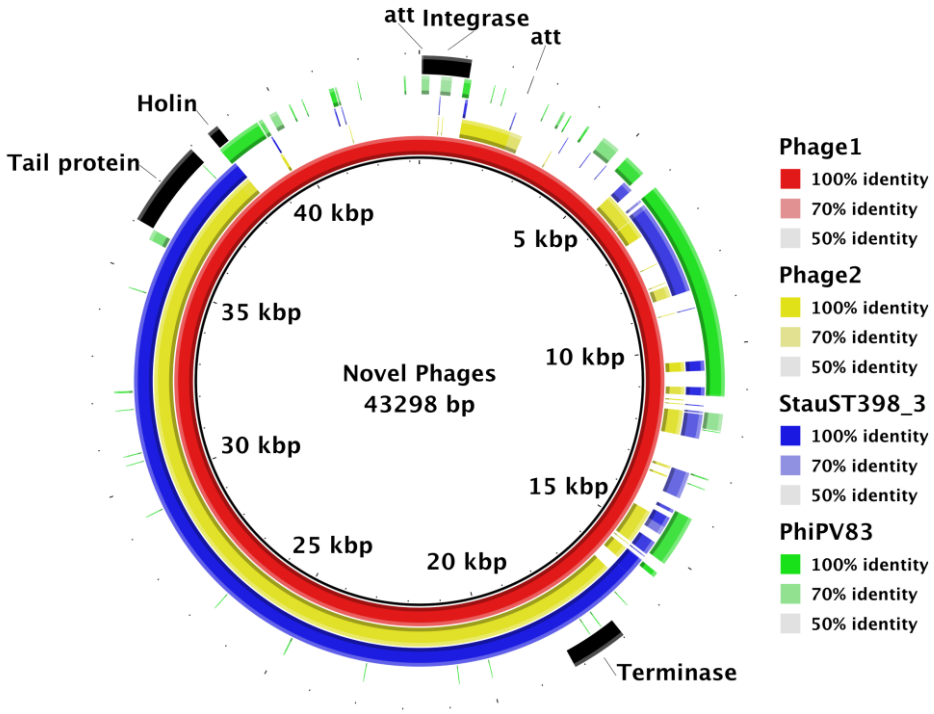


Figure 3.25. Comparison of the novel phages (phage 1 and 2) with previously published prophages DNA sequences.

3.7.2 CC398 Phylogeny based on whole genome sequencing

The phylogenetic tree was constructed based on the whole genome sequences alignment of the 28 CC398 isolates (the eight genome sequences (Table 3.8), and 20 further genome sequences from previously published study [190] (Table A.6). For SNPs calling, the 28 genome sequences were aligned against the published CC398 chromosome reference genome (strain S0385; accession no. AM990992). In addition, SNPs located in repetitive regions and mobile genetic elements such as (prophages, transposons, SaPIs and the SCC*mec* elements) were discarded. Hence, the SNPs alignment represented only the polymorphism within the core genomes of the investigated CC398 isolates. The phylogenetic tree was rooted using the *S. aureus* ST36 genome (accession no. BX571856) as outgroup (Figure 3.26).

We detected 1,656 SNPs, including 352 parsimony informative SNPs. Among these 1,656 SNPs, 1,604 were from coding regions (434 synonymous and 1,170 nonsynonymous) and 194 insertions or deletions ranging from 1 to 10 bp. Figure 3.26 represents the phylogenetic tree of the 29 CC398 (including the reference genome ST398_S0385) based on whole genome sequencing. The most ancestral lineage of CC398 composed of two MSSA isolates (LY19990171 and ST20091526) recovered from human and characterized by *spa* type t571 (Figure 3.26).

Of note, clade C was represented by six isolates (IMT26596, 11-00833, M2009_10004208, 06-02016, AV4 and 29139) (Figure 3.26); four of them were isolated from horses, while the remaining two isolates were environmental samples “pig (dust) [190]” (Figure 3.26, Table A.6). All these six isolates were characterised by SCC*mec* IVa. Clade C was defined by 14 SNPs (5 synonymous and 9 nonsynonymous) (Table 3.11).

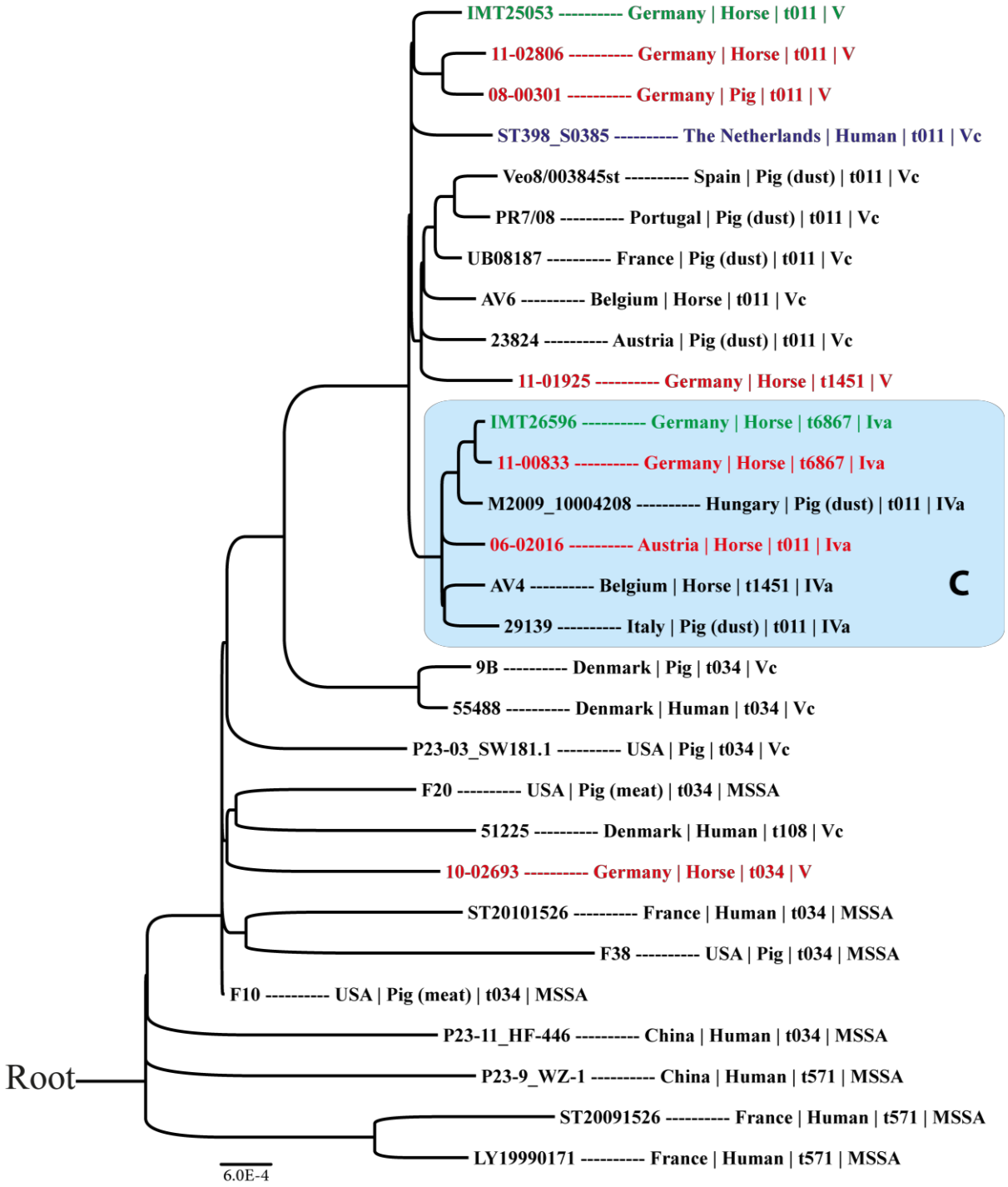


Figure 3.26. Maximum likelihood tree of 29 CC398 isolates (including the reference strain S0385) based on 1,656 SNPs, including 352 parsimony informative SNPs. Clade C is highlighted in lighten blue, reference strain genome (ST398_S0385) is dark blue, red labels represent isolates from Robert Koch Institute collection, while green labels represent isolates received from the Free University Berlin. All black-labelled isolates were included from previous study of Price et al. [190].

Table 3.11. Point mutations that define clade C based on whole genome sequencing.

SNP(s) position(s) in CC398 genome (strain S0385, accession number AM990992)	Ancestral	Derived	Quality	Locus	Product	Ancestral codon	Ancestral amino acid	Derived codon	Derived amino acid
34270	G	A	Synonymous	SAPIG0027	Hypothetical protein	GAG	Glutamine	GAA	Glutamine
34273	T	A	Synonymous	SAPIG0027	Hypothetical protein	GCT	Alanine	GCA	Alanine
111809	A	T	Non- Synonymous		No CDS is defined	AAG	Lysine	ATG	Methionine
1204607	C	A	Synonymous	SAPIG1148	Phosphodiesterase, family	GGC	Glycine	GGA	Glycine
1302177	G	A	Non- Synonymous	SAPIG1246	Succinate-CoA ligase, alpha subunit subfamily	GTT	Valine	ATT	Isoleucine
1320027	C	T	Non- Synonymous	SAPIG1264	RIP metalloprotease RseP	ACA	Threonine	ATA	Isoleucine
1326057	T	C	Synonymous	SAPIG1266	DNA polymerase III, alpha subunit, Gram-positive type	GTT	Valine	GTC	Valine
1477472	A	C	Non- Synonymous	SAPIG1402	Transcriptional regulator	AAT	Asparagine	CAT	Histidine
1517268	G	T	Non- Synonymous	SAPIG1434	EbhA protein	AGC	Serine	ATC	Isoleucine
1837869	G	T	Non- Synonymous	SAPIG1748	Isocitrate dehydrogenase, NADP-dependent	TAG	Stop codon	TAT	Tyrosine
1918655	C	T	Non- Synonymous	SAPIG1809	LPXTG-motif cell wall anchor domain	GCC	Alanine	GTC	Valine
2533404	A	T	Non- Synonymous	SAPIG2453	Nitrite-reductase [NAD(P)H], large subunit	AAC	Asparagine	TAC	Tyrosine
2724179	C	T	Synonymous	SAPIG2634	NAD-binding site protein	GTC	Valine	GTT	Valine
2843598	G	A	Non- Synonymous	SAPIG2732	Polysaccharide deacetylase domain protein	GTC	Valine	ATC	Isoleucine

4 DISCUSSION

4.1 Molecular epidemiology of CC398

In this study, a collection of 195 *S. aureus* CC398 isolates was investigated. This isolate collection was isolated between 1993 and 2011; recovered from 11 various host species and represented 10 different countries. Hence, this isolate collection represented a broad range of the CC398 population.

Pulsed Field Gel Electrophoresis (PFGE) using is considered to be the gold standard for the molecular typing of *S. aureus* isolates. However, CC398 was non-typeable by conventional *Sma*I-PFGE due to methylation of the *Sma*I site. Therefore, *spa*, MLST and SCC*mec* typing methods were considered as standardised tool for the epidemiological characterization of CC398 and other *S. aureus* lineages [234–236].

The molecular typing results suggest that the genetic diversity among the *spa* and SCC*mec* types of CC398 is limited. In addition, these findings are in good agreement with previous studies, which demonstrated that the *spa* types t011 and t034, and SCC*mec* types (IV and V) were the most common among CC398 isolates [145,149,237,238].

The antimicrobial resistance analysis of the 195 CC398 isolates showed that most of the isolates were mainly resistant to penicillin, tetracycline and oxacillin (Figure 3.3, Table A.1). Previous studies have reported that all LA-MRSA CC398 were tetracycline resistance [145,239]. Therefore, it has been suggested that the use of antimicrobial agents, mainly tetracycline, might be one of the reasons for the emergence of MRSA CC398 [240]. Furthermore, it was previously speculated that the tetracycline resistance phenotype could be used as an epidemiological marker for the LA-MRSA CC398 isolates [190,241]. However, the two tetracycline resistance genes *tet*(K) and *tet*(M) are located on plasmid (pT181) and transposon (*Tn*916), respectively; which was detected in methicillin-susceptible *S. aureus* [242], suggesting that tetracycline resistance cannot be the only factor responsible for the successful spread of CC398 between livestock. In addition, another study has demonstrated that tetracycline resistance phenotype is a misleading marker for the molecular genotyping of CC398

[241]. Previous studies have shown that the CC398 isolates carried novel multidrug resistance genes that were located on plasmids; including the trimethoprim and oxazolidinones resistance genes [187,243]. Taken together, these data might emphasise the high rate of horizontal transfer of genetic material, such as plasmids and transposons, from other bacteria to CC398.

4.2 Microevolution of CC398

The mutation rate of CC398 isolate collection was relatively faster compared to a previously reported evolutionary rate for other *S. aureus* clonal complexes [94,108]. These comparisons emphasise the variation of mutation rates among closely related *S. aureus* clones that may reflect variations in selective pressures faced in diverse ecological niches.

Based on the calculated mutation rate, the sequences variations and the isolation date of the 195 investigated CC398 isolates dataset (1993 – 2011); the time to most recent common ancestor was traced back to ≈ 1974 (95% confidence interval, 1955 to 1991). Of note, CC398 was non-typeable by *Sma*I-PFGE, so it is reasonable that CC398 has spread between animals for several years before they were first described to cause clinical infections between farmers [169]. Therefore, retrospective studies on the ancient non-typeable *S. aureus* strains using the recent molecular typing methods (e.g. MLST and *spa* typing) can lead to identify the basal CC398 isolates. Based on whole genome sequencing (WGS), a previous study showed that CC398 has originated in human and later transmitted to livestock [190]. However, this study did not estimate the date of the most-recent human ancestor of CC398. Although the CC398 isolates investigated here were collected over 18 years, they represented mainly the time period from 2006 to 2011 and some years were represented only by few isolates (e.g. 1993, n = 1; 2001, n = 1; 2002, n = 2). Applying WGS on a wider CC398 isolate collection that includes ancient and recent serially sampled isolates will precisely estimate the time to most recent common ancestor of CC398, and reduce the estimated confidence interval (1955 - 1991).

4.3 Phylogeny and population structure of CC398

The 96 detected SNPs within the screened 97 housekeeping loci of the 195 investigated CC398 isolates were used to construct the MST and to elucidate the phylogeny of CC398. The 195 CC398 isolate collection showed very limited diversity over the MST (Figure 3.5). However, the phylogenetic tree resolved CC398 into six distinct clades (Figure 3.6).

4.3.1 Phylogeography of CC398

The investigated 195 CC398 isolates represented ten different countries. Among these ten countries, origin from six European countries was significantly associated with phylogeny (Table 3.2), which suggests the wide dissemination of CC398 in various countries. Of note, CC398 has first been detected in Europe [169], and later reported in several other geographic regions worldwide such as North and Central America, Singapore, China, Australia and New Zealand [170,171,244–246]. In addition, it was shown that CC398 isolates from different countries were similar in terms of their genetic content [247]. Of note, food animals are exported around the world and CC398 may have deposited away inside them. Similarly, a previous study has revealed that the spread of the poultry *S. aureus* ST5 lineage was strongly consistent with the globalized nature of the poultry industry [163]. Taken together, these data emphasize the potential impact of globalization and the livestock trade routes for the emergence and spread of CC398 worldwide.

However, the investigated CC398 isolate collection contained few countries that were inadequately represented such as Canada (n = 1), Thailand (n = 1) and Italy (n = 3). For in-depth investigation of the CC398 phylogeography, it would be necessary to include demographically and geographically representative isolates of further geographical origins (e.g. China, Australia, New Zealand and African countries).

4.3.2 The phylogenetic analysis emphasise the limitations of *spa* and SCC*mec* typing approaches

Rooting the CC398 phylogenetic tree using the concatenated sequences of the investigated isolates and N315 as an out-group showed that a cluster of two isolates, characterised by the *spa* type t899, was the most divergent compared to the remaining CC398 isolates (Figure 3.8). The SNPs discovery using dHPLC revealed that t899 isolates had 10 SNPs in three different housekeeping loci (au200, au201 and au202), which were located on the isolates chromosomes with a region of ≥ 111,139 bp (between 23,209 – 134,348) (Table A.7). These findings are in agreement with a study based on whole genome sequencing, suggested that CC398 with *spa* type t899 had acquired a fragment of 123,000 bp from ST9 through horizontal gene transfer. This fragment included the *spa* gene and the SCC*mec* insertion site [190].

The *S. aureus* clone (ST9) was detected in pigs and other livestock worldwide [184]. In *S. aureus*, variation of *spa* types has been described previously as a result of replacement of a large chromosomal DNA fragment from other lineages. For instance, the epidemic *S. aureus* clone ST239 has emerged likely after such recombination event between ST8-like and ST30-like chromosomes [121]. Hence, *spa* typing has limitations, and MLST typing is required for accurate identification of the t899 isolates. In addition, homoplasy within the *spa* gene might lead to discrepancies between the phylogeny of CC398 and *spa* types. Previous study demonstrated that *spa* typing can misclassify specific *S. aureus* lineages due to recombination events and homoplasy and might occasionally lead to misinterpretation [94,106,190].

In this study, MRSA CC398 isolates characterised by SCC*mec* types IV and V and the methicillin-susceptible CC398 isolates were significantly associated with phylogeny (Table 3.4). Most MSSA CC398 isolates were traced adjacent to the root of the phylogenetic tree. These findings are in agreement with previous CC398 study, which suggested that CC398 had originated as MSSA and afterward acquired the resistance to methicillin [190]. In addition, these data may suggest that CC398 encountered selective pressure through the antibiotic usage that led to the acquisition of SCC*mec* element.

The horizontally transferred region observed among the t899 of ST9 and CC398 isolates also carries the SCC*mec* element, which suggests an alternative mechanism for SCC*mec* dissemination between *S. aureus* clones. Taken together, these data strongly support previous findings that the dissemination of SCC*mec* elements in MRSA lineages has occurred on several occasions [94,248].

4.4 Human-to-livestock host jumps

The 195 CC398 isolate collection investigated in this study was collected from 11 host species (Table A.1). Human (n = 80) and animals (n = 115) CC398 isolates were isolated from colonisation and infection sites. CC398 was previously detected in multiple host species [141]. Previously, Price et al [190] has shown that CC398 originated in human as MSSA and subsequently spread to livestock, where it acquired resistance to methicillin and tetracycline. In addition, human MSSA isolates with *spa* type t571 were the most ancestral lineage on the CC398 phylogenetic tree that was based on whole genome sequencing [190]. In this study, the phylogenetic tree based on dHPLC approach revealed that the entire MSSA CC398 isolates from human with *spa* type t571 (n = 5) were located near to the root (Figure 3.8). In addition, MSSA CC398 isolates from both human and animals with *spa* type t034 were also located near to the root. Nevertheless, the dHPLC-based mutation discovery method applied in this study covered 1.4% of the CC398 genome; therefore, the CC398 phylogenetic tree did not reflect the human-to-animal host jumps that accomplished CC398. In addition, human CC398 isolates were distributed on the phylogenetic tree (Figure 3.10) and were not significantly different from a random distribution ($p > 0.1$; Table 3.5).

In contrast to the LA-MRSA-CC398 isolate, the human-associated MSSA CC398 isolates harboured the β- converting φSa3 prophage, which carries genes (*sak*, *chp* and *scn*) associated with human innate immunomodulation [190]. It was shown that φSa3 prophage plays an important role in the adaptation of *S. aureus* to the human niche [79,192]. The loss of φSa3 prophage in LA-associated isolates, hence, suggests adaptation of CC398 to the animal-niche [190]. In agreement with the observation of Price et al., the immune evasion genes (*sak*, *chp* and *scn*) carried by the φSa3 prophage were detected in 17 CC398 isolates (Figure 3.11.A). However, only eleven isolates were of human origin, while six were of animal origin (Table A.9). All the human

isolates that carried φSa3 with exception of one were MSSA, while the animal isolates were all MRSA. These findings suggest that φSa3 prophage was acquired by CC398 through horizontal gene transfer.

We observed that MSSA CC398 isolated from turkey meat (n = 4) shared the same point mutations (Figure 3.10), and they were significantly associated with phylogeny (Table 3.5). The φAvβ prophage sequences were detected in these four CC398 isolates using PCR approach (Table A.9). This φAvβ prophage was previously detected in *S. aureus* CC5 from various bird species, and in industrially fattened broiler chickens [163]. The φAvβ prophage carries avian-niche-specific genes, which suggests that MSSA CC398 in turkey flocks have adapted to the host through acquisition of an avian-specific prophage, similar to *S. aureus* CC5 in industrially fattened broiler chickens [163]. This explanation is in agreement with previous CC398 study based on whole genome sequencing [190].

Only handful of breeder companies is responsible for turkey production worldwide [249]. Furthermore, the antimicrobials abuse at farms is considered as a risk factor for the wide dissemination of MRSA between animals [250]. In the United States, the Union of Concerned Scientists demonstrated that more than 70% of all sold antibiotics were given during fattening to the livestock; including swine, cattle and poultry [251]. MRSA CC398 was detected between turkey flocks, and people in frequent contact with them [252,253]. Both ST5 and CC398 were the most dominant *S. aureus* between chicken and turkey [254], which possibly enhanced the horizontal gene transfer between both lineages.

4.5 Immune evasion cluster genes protect CC398 against phagocytosis

As revealed previously, there is increasing evidence for an evolutionary trend of increasing pathogenicity of CC398 for humans. One example of this is the acquisition of the φSa3 prophage, which carries so-called immune evasion cluster (*sak*, *chp* and *scn*) genes [190,192].

In this study, the CC398 positive to the immune evasion cluster genes were significantly less engulfed by human granulocytes, after 30 and 60 min, compared with their negative counterparts (Figure 3.18). These findings indicate the significant

contribution of the immune evasion cluster genes to the protection of CC398 from the human immune response. This would explain the ability of CC398 to invade the human immune system and then disseminating systemically to cause severe infection. It was shown that the immune evasion cluster genes produce proteins that hamper the phagocytosis of other *S. aureus* strains by human neutrophils [255]. In addition, previous studies revealed selection against the immune evasion cluster in animal hosts, which was suggested as a part of CC398 adaptation to non-human hosts [190,247]. However, all the investigated CC398 isolates were collected from both human and animal hosts (Table 3.7). A recent study revealed that CC398 isolates with *spa* type t108 were significantly less engulfed by human granulocytes compared with t011, t034 and other CA-MRSA isolates [256]. Nevertheless, only one CC398-t108 isolate (07-02239) was included here that carried the immune evasion cluster genes (Table 3.7). Hence, CC398-t108 is unlikely to have played a part in the significant reduction in the phagocytosis of CC398 that was observed in our study.

Several studies revealed that *S. aureus* strains harboured the PVL genes were able to evade the human immune system causing severe infections [74–77]. PVL toxin is able to lyse the mammalian leukocytes, which are involved in the phagocytosis of *S. aureus*, by forming pores in their cell membrane. To exclude the effect of PVL as putative immune evasion factor, all the investigated CC398 isolates were PVL negative. Hence, the difference observed in the CC398 isolates' evasion capacity to the human granulocytes could not be explained by the expression of PVL toxins.

Taken together, these data show for the first time that CC398 can hamper the phagocytosis by human granulocytes. However, these findings might not translate directly to human adaption of CC398. In contrast to well-studied intracellular pathogens and other *S. aureus* lineages, nothing is yet known about how CC398 can survive phagocytosis. For instance, it was previously shown that the presence of iron-regulated surface determinant protein might reduce the uptake of *S. aureus* by human neutrophils [257]. In addition, some *S. aureus* strains can survive in the human macrophages by secreting proteins such as metalloprotease, which resist the human antimicrobial peptide [258]. Further research needs to be performed to investigate the expression levels of the immune evasion cluster genes in the CC398 isolates from dif-

ferent host species, which might be a promising approach for explaining this observation in more details.

4.6 Emergence of equine-associated CC398 sub-clone

Interestingly, clade C consisted mainly of isolates from horses under veterinary care (n = 41). In total, 53 CC398 isolates from horses were included in the present study. Isolates from other host species (Figure 3.10) had been also allocated in clade C; however, the epidemiological data shown that those hosts were in close contact with infected horses (Table A.1). Isolates in clade C (n = 53) were collected from four different countries (Figure 3.7) between 2006 and 2011. These findings suggest that clade C is a distinct sub-clone within CC398, which is disseminated among hospitalised horses and veterinary personnel all over Germany and in several European countries. Of note, the majority of clade C isolates were characterized by *spa* type t011 (95%) and SCC*mec* IV (91%) (Table A.1).

Several studies showed that MRSA CC398 with *spa* type t011 and SCC*mec* type IV was the most dominant strain among the MRSA infections and carriage in horses in Europe. For instance, in the Veterinary University of Vienna, several cases (n = 9) have been detected [244]. In Belgium, screening of horses on admission to a Belgian equine clinic revealed a high colonization percentage with MRSA-CC398-t011-IV [259]. The same MRSA CC398 sub-clone was detected among horses in a veterinary hospital in Finland between 2007 and 2009; subsequently, it caused infection in one employee of the stuff members [260]. The first report of CC398 from England was in 2009; there the MRSA-CC398-t011-IV was detected in two horses admitted to the Equine Referral Hospital at the Royal Veterinary College [261]. Furthermore, a previous study, based on MLST, *spa*- and SCC*mec* typing, has shown that MRSA-CC398-t011-IV caused nosocomial infections in horses in an equine clinic in Switzerland [262]. The authors reported that the same CC398 strain was initially detected in one of the personnel members who formerly worked in an equine clinic in Belgium. Later, this MRSA-CC398-t011-IV was detected in infected horses and subsequently replaced ST1-t2863, which was dominant in wound infections in this equine clinic [262]. These data suggest that all the detected CC398 strains from those studies [244,259–262] were likely affiliated to clade C. In addition, isolates from clade C commonly

showed resistance to gentamicin, which is the first choice antibiotic and frequently used in the treatment of infections in horses [263–265]. This finding suggests that CC398 encountered selective pressure for gentamicin resistance in equine settings, which is comparable to the epidemic EMRSA-15 strains that encountered selective pressure for clindamycin and erythromycin resistance in veterinary and human healthcare settings in the United Kingdom; respectively [266]. In addition, the authors suggested that the reason for this selection in EMRSA-15 strains was due to the wide use of clindamycin and erythromycin in veterinary practice and human medicine in the United Kingdom; respectively [266]. Worth mentioning that clade C was extremely rare among *S. aureus* isolates from human infections in Germany, and its association with infections in horses was highly significant ($p < 0.0001$). Taken together, these findings raised the debate whether the emergence of that MRSA-CC398-t011-IV reflects epidemic spread or host adaptation?

4.6.1 Equine CC398-host interactions

In order to test the hypothesis whether the equine CC398 sub-clone is better adapted to horses than to other host species, we studied phenotypic characters of this equine CC398 isolates, including their adhesion capacity to host fibronectin. The ability of *S. aureus* to interact with the host's plasma fibronectin is essential for host colonization and infection. The CC398 isolates set, including the equine CC398 isolates (MRSA-CC398-t011-IV), demonstrated no significant differences in the adherence ability to both human and equine fibronectin, suggesting that differences in the host origin of fibronectin do not play a significant role in the adhesion of CC398. These observations are in agreement with a previous study, which showed no significant differences between the binding ability of CC398 to the human and bovine plasma fibronectin [256]. Another study showed that the adhesion capacity of LA-CC398 to the human and porcine keratinocytes did not differ [192]. However, only isolate 10-02693 (horse-t034-V) adhered significantly more efficient to the equine fibronectin compared with its human counterpart (Figure 3.13). Since it was reported that LA-CC398 isolates harboured point mutations in several genes that encode adhesion factors [192], therefore, we compared the sequences of the genes encoding the fibronectin-binding proteins (*fnbA* and *fnbB*). This comparison revealed that all the investigated CC398 isolates, with exception of isolate 10-02693, harboured a point mutation

in *fnbB* gene similar to that found in the CC398 reference genome (strain S0385), which lead to truncation in the encoded protein [192] (Table 3.10). This may likely explain the adhesion differences of isolate 10-02693 to both human and equine fibronectin. Furthermore, these findings indicate that the equine MRSA-CC398-t011-IV sub-clone is likely able to initiate colonization in exposed human and equine. However, further investigations are needed to determine the *fnbB* gene expression of these individual CC398 isolates. Several *S. aureus* surface proteins mediate its adhesion to the host tissue; therefore, investigating the adhesion of the CC398 to the human and equine nasal mucosa and keratinocytes might deliver a better understanding of the emergence of MRSA-CC398-t011-IV sub-clone between different hosts.

4.6.2 Equine CC398 sub-clone is not generally protected against phagocytosis

Another approach for studying the attributes of equine CC398 was to investigate its ability to invade different host species by escaping their innate immune responses. We observed that human and horse granulocytes had similar capacity to phagocyte CC398 isolates, which may reflect the narrow spectrum of virulence characteristics of CC398 and its limited level of host specificity. In addition, the results showed significant increased phagocytosis toward equine CC398 isolates and isolate 10-02693 by horse granulocytes, which may suggest that the phagocytosis of CC398 by horse granulocytes is isolate dependent. One can speculate that equine CC398 sub-clone is not generally protected against the phagocytosis by horse granulocytes. Interestingly, the horse monocytes showed significantly higher uptake of CC398 compared with its human and pig counterparts, which might suggest that horse monocytes plays an important role in the elimination of invading pathogens. However, little is known about the putative role of horse monocytes in phagocytosis and it remains to be elucidated.

Pig neutrophils had significantly lower uptake capacity of CC398 compared to their human and horse counterparts, which may likely suggest the enhanced clearance capacity of CC398 by pig neutrophils. Previous studies showed that the porcine genes encoding toll-like receptors, key components of the host innate recognition system, harboured polymorphisms compared to their human counterparts [267,268]. Furthermore, It was suggested that this maintenance of diversity in the toll-like receptors genes might have some advantages for the pig's immune system to eliminate

pathogens efficiently [267–269]. Another explanation could be the use of antibiotics as a growth promoter in conventionally raised pigs such as tetracycline, which was previously shown to decrease the efficiency of neutrophils phagocytic activities [270,271]. However, the degree of uncertainty in these estimates is considerable, and no firm conclusions should be drawn. The phagocytosis assay used in this study can only reflect an approximation to *in vivo* phagocytosis and cannot clarify the observed reduction in the uptake of CC398 by pig neutrophils, especially since there are several factors involved in the host innate immune response. For instance, the hosts' neutrophils employ several oxygen-dependent and oxygen-independent strategies to eliminate the invading pathogens. Although achievements have been made concerning understanding mechanisms used by CC398 to evade host's innate system, particularly those including neutrophils, our understanding in this area still incomplete.

4.7 Whole genome sequencing and the nosocomial spread of equine CC398 lineage

The dHPLC-based mutation discovery approach applied in this study delivered improved discriminatory power compared to *spa* typing and the MLST typing methods, and provided several novel insights into *S. aureus* population structure [94,107,135]. However, it covered only 1.4% of the CC398 genome, and clade C was defined by one synonymous base substitution. Hence, the resolution of analyses and the strength of any inferences would be much improved by whole-genome-sequencing [35,108].

The phylogenetic tree of the 28 CC398 isolates based on whole genome sequencing, presented in this study, confirmed the emergence of clade C among horses and was defined by 14 point mutations. The isolates that formed clade C on this phylogenetic tree were all isolated from horses, with exception of two isolates (29139 and M2009_10004208) that were originating from pig (dust). These two sequences were included from a previous study [190], which did not provide any further epidemiological data concerning the previous contact with horses. The fact that *spa* type t011 and SCC*mec* IV characterized those environmental isolates, likely suggests their previous contact with horses.

As the equine MRSA-CC398-t011-IV sub-clone appears to have become established within the equine settings; however, our understanding of the genetic determinants of this equine CC398 sub-clone remains incomplete. In addition, a better understanding of the features that make this equine CC398 sub-clone successful within and among equine settings, from several countries, was urgently required. To address this issue we used a comparative genomics approach that involved whole genome sequencing of eight CC398 isolates, including three isolates from clade C. The isolates chosen here for sequencing were phylogenetically divergent, thus raising the amount of informative data produced. The most striking finding of the comparative genomics approach is that three CC398 horse isolates, which do not belong to clade C, harboured a novel pathogenicity island that share DNA sequences similarities with SaPIbov4. These data emphasise the importance of horizontal gene transfers in the genetic diversity of CC398. In addition, the observed divergence of *vwb* genes sequences carried by SaPIbov4-like might reflect an additional strategy for the virulence of CC398.

The detection of novel phages that are harboured by different isolates that were belonging to different clades suggests that several phages are associated to CC398, and they may have played a role for its evolution. The DNA sequences of these phages encode functional gene products that are required for the phage propagation. Extensive experiments need to be performed to investigate whether these two prophages might influence the virulence of CC398. However, it was claimed that bacteriophages might influence the adaptation of CC398 to different host-niches through several mechanisms; including the carriage of genes that encode immune evasion modulators [190]. As these novel phages had approximately similar size and were closely related across their genome, so one can speculate that these phages were acquired independently by each isolate through horizontal gene transfer.

One of the most significant findings here is that clade C isolates genomes showed high levels of similarities to the LA-CC398 reference genome (strain S0385), including the majority of surface proteins, the mobile genetic elements and the lack of virulence factors such as enterotoxin and exfoliative toxin genes. These findings suggest that the equine CC398 sub-clone had not encountered better adaptation to equine. Furthermore, one can speculate that the genomic content of this equine CC398

sub-clone is adequate for successful dissemination of this isolates between different host species. Similarly, a recent study, based on whole genome sequencing, has shown that EMRSA-15 strains isolated from human did not differ compared to their counterparts from companion animals concerning their genomic content [266]. In addition, the authors suggested that the ability of *S. aureus* lineages to infect an extended host spectrum might be distinctive for their long-term evolutionary history [266].

Taken together, the emergence of this MRSA CC398 sub-clone in horses from several European equine clinics is likely due to an epidemic spread, which is comparable to several epidemic MRSA strains that rapidly spread within and between medical care hospitals and cause a large number of healthcare-associated infections in humans [35,272,273]. The spread of this equine CC398 sub-clone might be due to insufficient hygiene practices in veterinary settings. Several studies have reported that the nasal carriage rate of MRSA among veterinary practitioners is much greater than in medical staff in human hospital [274–277]. Furthermore, the nosocomial spread of MRSA in equine clinics and among veterinary personnel was previously demonstrated [144,182,278–283]. Hence, personnel in veterinary settings may play an important role in the introduction and spread of MRSA into equine clinics. In addition, humans with frequent contact with horses can represent a reservoir for MRSA and then transmit it to their household. A metapopulation model showed that the occurrence of a relatively large proportion of MRSA-CC398 carriers between susceptible human populations might result in an outbreak [284]. One can speculate that the direct transmission between horses should be easier to eliminate in equine compared with human hospitals, as horses are commonly not allowed to move freely in the equine hospitals. Insufficient hand hygiene is a key route for MRSA transmission within equine hospitals [147]; therefore, the implementation of good hand hygiene practice is the significant measure for preventing nosocomial infections.

4.8 Conclusions and future perspectives

In this study, we demonstrated a new insight into the phylogeny of CC398 based on genome-wide mutation discovery. We revealed the dissemination of a specific MRSA-CC398 sub-clone "dubbed clade C" within and between different equine settings, which caused infections in horses and nasal colonisation of humans. Based on the phenotypic characterisation of this CC398 sub-clone, we could demonstrate the lack of host adaptation. Moreover, the genomic analysis presented here together with the epidemiological data, provide evidence that the spread of this equine CC398 sub-clone is due to epidemic spread. The spread of this equine CC398 sub-clone can be traced through testing for the presence/ absence of the SNPs defining the clade C (canonical SNPs) using diagnostic PCR followed by sequence analysis. Improvements in the infection control programmes by checking the efficacy of the methods of disinfection or sterilization may reduce the frequency of infection. Veterinarians play an important role in controlling the transmission of this sub-clone by taking precautions with staff hygiene, and implementation of risk reduction strategies for infection prevention.

In the last years, the comparative genomics studies provided important insights into genetic variation in the populations of *S. aureus* and demonstrated its power to address issues of evolution and host adaptation. The comparison of the genome sequences of CC398 isolates has led to some unpredicted discoveries about the number of pathogenicity islands that differ between closely related isolates, and the prevalence dynamics of phages. Overall, these findings were not only enhancing our understanding of the equine CC398 genomic contents, but also provided an insight into the evolution of CC398.

It will now be exciting to investigate the role of these novel mobile genetic elements in the virulence of CC398. This can be achieved by performing *in vitro* assays that measure the invasion of different host cells (e.g. epithelial and endothelial cells) by CC398 strains. Furthermore, one can make use of the available genome sequence data of CC398 to construct whole-genome microarrays. These microarrays will allow us to investigate the gene expression, and the interactions between thousands of genes in CC398 strains under different environmental growth conditions.

Alternatively the transcriptome of CC398 can be inferred and quantified by using the high-throughput next-generation RNA sequencing (RNA-seq). This RNA sequencing approach has several advantages over the microarray-based method; including the higher genome coverage, higher-sensitivity and it can capture nearly all of the expressed transcripts. The first functional genomics study on *S. aureus* N315, based on RNA sequencing, revealed approximately 200 novel transcripts [285]. Therefore, RNA sequencing is considered as a revolutionary tool for functional genomic research.

Various methods can be used to investigate how CC398 might spread among veterinary settings and across the borders. For instance, we could mine the epidemiological data together with phylogeographic analysis for additional insights relating to the identification of the international transmission routes for CC398. In addition, using wireless sensor implants combined with the Global Positioning System coordinates to track pets and livestock interactions and contact nodes will be a promising method for elucidating the transmission dynamics of CC398.

Several studies, based on metagenomic analysis, revealed that shifts in the composition of gut microbiota is associated with several diseases such as inflammatory bowel diseases, ulcerative colitis, and *Clostridium difficile* infections [286–289]. It will now be exciting to use metagenomic approaches for investigating whether the nasal microbiome composition differs in humans colonised and infected with CC398 compared with non-carriers. Such studies will allow us to better understand the interaction between CC398 and other bacterial species, and the relative abundance of certain bacterial species might help to develop new treatment strategies. For instance, probiotic-based approaches that restore the balance in the nasal microbiome composition might be promising strategies for the decolonization and treatment of CC398 positive patients; similar to those used to treat *Clostridium difficile* infections [290,291].

We should keep in mind that the frequent exposure of CC398 to antimicrobial agents, in livestock, increases the selection pressure for antibiotic resistance. Hence, an international multidisciplinary effort is needed to slow the development of antibiotic resistance through avoiding the overuse of antibiotics in livestock.

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A. APPENDIX

Table A.1. CC398 Investigated Isolates

Isolate ID	Synonym	Country	Farm/Stationary or Ambulatory Care**	Isolation date	*Spa* type	SCC*mec*	Host	Characteristic	Resistance*	Clade
04-03094†	MRSA	Germany	N.A.	01.06.2004	t034	V	Human		PEN,ERY, OXA, CLI, TET,	A
05-02057†		Germany	N.A.	01.06.2005	t034	V	Dog		PEN; ERY; CLI; TET	N.A.
06-00262†	E 64	Austria	S.C. 1	01.06.2006	t011	IV	Horse	Wound infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	N.A.
06-00880†		Austria	S.C. 1	01.06.2006	t011	IV	Horse	Infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
06-00903†	BK 201770	Germany	N.A.	13.04.2006	t011	V	Human	Bacteremia / Sepsis	PEN; ERY; CLI; TET; CIP	N.A.
06-02016†	Shila	Austria	S.C. 1	13.04.2006	t011	IV	Horse	Infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
06-02985†	M1960	Austria	N.A.	10.04.2006	t011	V	Human	Infection	PEN; TET; SXT	N.A.
06-03005†	242003	Germany	N.A.	01.12.2006	t571	MSSA	Human	Wound infection	ERY	N.A.
06-03034†	VA 318796	Germany	N.A.	10.12.2006	t034	V	Human	Commensal	PEN; OXA; ERY; CLI; TET; SXT	N.A.
07-00334†	M 25	Austria	S.C. 1	10.12.2006	t011	IV	Human	Commensal	PEN; OXA; GEN; TET; SXT	C
07-00415†	1000 4239	Germany	N.A.	01.06.2007	t034	MSSA	Human		PEN; TET	N.A.
07-00471†	M 34	Austria	S.C. 1	01.06.2007	t011	IV	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-00755†	W 372/06	Austria	S.C. 1	01.06.2007	t011	IV	Horse	Wound infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-00757†	W 1129/06	Austria	S.C. 1	01.06.2007	t011	IV	Horse	Wound infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01238†	H 99	Austria	S.C. 1	01.06.2007	t011	IV	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01239†	H 101	Austria	S.C. 1	01.06.2007	t011	IV	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01274†	159	Germany	N.A.	26.04.2007	t034	V	Pig		PEN; OXA; ERY; CLI; TET; SXT	A
07-01335†	H 73	Austria	S.C. 1	26.04.2007	t011	IV	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01337†	R 109	Austria	S.C. 1	01.06.2007	t011	IV	Horse	Infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01388†	E 120	Austria	S.C. 1	01.06.2007	t011	IV	Horse	Infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01429†	2451294	Germany	N.A.	01.06.2007	t571	MSSA	Human	Wound infection	ERY	N.A.
07-01494†	235633	Germany	N.A.	01.06.2007	t034	V	Human	Wound infection	PEN; OXA; ERY; CLI; TET	A
07-01640†	235633	Germany	N.A.	25.05.2007	t034	V	Human	Wound infection	PEN; OXA; ERY; CLI; TET	A

07-01653†	VA 9476872-WA	Germany	N.A.	24.05.2007	t034	V	Human	Wound infection	PEN; OXA; ERY; CLI; TET; CIP; SXT; MFL	E
07-01730†	H120	Austria	S.C. 1	01.06.2007	t011	IV	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-01826†	81	Germany	N.A.	01.06.2007	t1197	MSSA	Human	Wound infection	TET; CIP; SXT; MFL	N.A.
07-01949†	E 130	Austria	S.C. 1	01.06.2007	t011	IV	Horse	Infection	PEN; OXA; GEN; ERY; CLI; TET; SXT	C
07-02239†	VA 312822	Germany	N.A.	01.06.2007	t108	V	Human	Wound infection	PEN; OXA; TET; CIP; MFL	B
07-02347†	88 732B 755	Germany	N.A.	01.06.2007	t034	nt	Human	Commensal	PEN; OXA; ERY; CLI; TET	A
07-02415†	1	Denmark	N.A.	01.06.2007	t2974	V	Pig		PEN; OXA; TET	N.A.
07-02424†	11	Denmark	N.A.	03.09.2007	t011	V	Pig		PEN; OXA; GEN; ERY; CLI; TET; SXT	D
07-02431†	H2	Germany	N.A.	03.09.2007	t011	V	Human		PEN; OXA; GEN; ERY; CLI; TET; SXT	D
07-02432†	H3	Germany	N.A.	03.09.2007	t011	V	Human		PEN; OXA; GEN; ERY; CLI; TET; SXT	D
07-02433†	H4	Germany	N.A.	03.09.2007	t011	V	Human		PEN; OXA; GEN; ERY; CLI; TET; SXT	D
07-02464†	H12	Germany	N.A.	03.09.2007	t034	MSSA	Human		PEN; ERY; CLI; TET; SXT	F
07-02558†	88 735B 930	Germany	N.A.	03.09.2007	t034	V	Human	Abscess	PEN; OXA; ERY; CLI; TET	A
07-02632†	H 824	Germany	N.A.	03.09.2007	t034	V	Human		PEN; OXA; ERY; CLI; TET; SXT	A
07-02642†	H 827	Germany	N.A.	10.09.2007	t034	V	Human		PEN; OXA; ERY; CLI; TET; SXT	F
07-03026†	25533	Germany	N.A.	03.09.2007	t011	V	Human	Wound infection	PEN; OXA; TET	N.A.
07-03443†		Germany	N.A.	03.09.2007	t034	V	Pig		PEN; OXA; ERY; CLI; TET	A
08-00301†	B 11	Germany	N.A.	03.09.2007	t011	V	Pig		PEN; OXA; GEN; ERY; CLI; TET; SXT; TGC	D
08-00306†	H 2	Germany	N.A.	30.01.2008	t011	V	Human		PEN; OXA; ERY; CLI; TET; TGC	D
08-00307†	H 3	Germany	N.A.	30.01.2008	t011	V	Human		PEN; OXA; ERY; CLI; TET; TGC	D
08-00308†	H 4	Germany	N.A.	30.01.2008	t011	V	Human		PEN; OXA; GEN; ERY; CLI; TET; SXT; TGC	D
08-00360†	H 6	Germany	N.A.	30.01.2008	t034	MSSA	Human		PEN; TET; SXT; TGC	F
08-00401†	16	Germany	N.A.	30.01.2008	t034	V	Pig		PEN; OXA; ERY; CLI; TET; SXT; TGC	N.A.
08-00537†		Germany	N.A.	30.01.2008	t034	MSSA	Turkey	Skeletal infection	PEN; ERY; CLI; TET; CIP	N.A.
08-00543†		Germany	N.A.	01.06.2008	t034	MSSA	Turkey	Skeletal infection	PEN; TET	N.A.
08-00700†	H1	Germany	N.A.	01.06.2008	t011	MSSA	Human		PEN; ERY; CLI; TET	N.A.
08-00798†		Germany	N.A.	01.06.2008	t034	MSSA	Turkey	Skeletal infection	PEN; ERY; CLI; TET	N.A.
08-00888†		Germany	N.A.	01.06.2008	t034	MSSA	Turkey	Skeletal infection	PEN; TET; CIP	N.A.

08-00907†	H 1	Germany	N.A.	01.06.2008	t011	V	Human		PEN; OXA; ERY; CLI; TET	N.A.
08-00912†	S 5	Germany	N.A.	01.06.2008	t034	V	Pig		PEN; OXA; ERY; CLI; TET; SXT	E
08-01058†	H 4	Germany	N.A.	01.06.2008	t034	MSSA	Human		PEN; TET; SXT	N.A.
08-01223†	H 4,1	Germany	N.A.	01.06.2008	t034	MSSA	Human		PEN; TET; SXT	N.A.
08-01388†	VA 309257	Germany	N.A.	01.06.2008	t011	V	Human	Wound infection	PEN; OXA; TET	N.A.
08-01605†	H 800/2	Germany	N.A.	01.06.2008	t011	MSSA	Human		PEN; ERY; CLI; TET; SXT	N.A.
08-01712†	S 1a	Germany	N.A.	01.06.2008	t011	V	Pig		PEN; OXA; TET; SXT	N.A.
08-01737†	H 28 S 3	Germany	N.A.	01.06.2008	t011	V	Pig		PEN; OXA; TET; SXT	N.A.
08-01849†		Germany	N.A.	01.06.2008	t011	V	Human		PEN; OXA; TET	N.A.
08-02208†		Germany	N.A.	06.10.2008	t034	V	Human		PEN; OXA; ERY; CLI; TET	E
08-02418†	E 855	Germany	N.A.	06.10.2008	t034	V	Human	Wound infection	PEN; OXA; ERY; CLI; TET	F
08-02710†	339	Germany	N.A.	06.10.2008	t034	MSSA	Human		PEN; ERY; CLI; TET	A
08-02807†	579	Germany	N.A.	06.10.2008	t034	V	Human		PEN; ERY; CLI; TET	F
09-00339†	2006S318	Belgium	N.A.	06.10.2008	t011	V	Human		PEN; OXA; ERY; CLI; TET; CIP; SXT; MFL	N.A.
09-00340-1†	2006S488	Belgium	N.A.	06.10.2008	t011	IV	Horse		PEN; OXA; GEN; ERY; CLI; TET; SXT	C
09-00341†	2007S256	Belgium	N.A.	06.10.2008	t011	V	Pig		PEN; OXA; TET; CIP; MFL	N.A.
09-00342†	2007S174	Belgium	N.A.	06.10.2008	t011	V	Chicken (thaw water)		PEN; OXA; ERY; CLI; TET	N.A.
09-00343†	2007S413	Belgium	N.A.	06.10.2008	t011	V	Horse		PEN; OXA; TET	N.A.
09-00393†	1	Germany	N.A.	01.06.2009	t034	V	Human	Commensal	PEN; OXA; ERY; CLI; TET	N.A.
09-00443†	H2	Germany	N.A.	01.06.2009	t011	V	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	D
09-00444†	H3	Germany	N.A.	01.06.2009	t011	V	Human	Commensal	PEN; OXA; GEN; ERY; CLI; TET; SXT	D
09-00445†	H4	Germany	N.A.	01.06.2009	t011	V	Human	Commensal	PEN; OXA; ERY; CLI; TET; SXT	D
09-00709†	1	Germany	N.A.	01.06.2009	t034	V	Human	Commensal	PEN; OXA; ERY; CLI; TET	E
09-00711†		Germany	N.A.	01.06.2009	t034	V	Pig	Commensal	PEN; OXA; ERY; CLI; TET	N.A.
09-01308†	SW 61.2	USA	N.A.	01.06.2009	t034	V	Pig		PEN; OXA; TET	N.A.
09-01309†	SW 181.1	USA	N.A.	01.06.2009	t034	V	Pig		PEN; OXA; ERY; CLI; TET	N.A.
09-01310†	SW 31.1	USA	N.A.	01.06.2009	t034	V	Pig		PEN; OXA; TET	N.A.

09-01311†	05.9498.K	UK	N.A.	22.10.2005	t034	MSSA	Human	Sepsis	PEN	N.A.
09-01312†	NY.ST398	USA	N.A.	01.06.2009	t571	MSSA	Human		PEN; ERY	N.A.
09-01313†	07.6579.J	UK	N.A.	23.08.2007	t034	V	Environment		PEN; OXA	N.A.
09-01314†	07.8588.D	UK	N.A.	12.11.2007	t034	V	Human	Wound infection	PEN; OXA	N.A.
09-01315†	07.2736.J	UK	N.A.	05.03.2007	t034	V	Human	Infection	PEN; OXA	N.A.
09-01316†	HU 01010T	USA	N.A.	01.06.2009	t034	V	Human		PEN; OXA; TET	N.A.
09-01318†	07.7640.J	UK	N.A.	04.10.2007	t034	V	Human	Infection	PEN; OXA	N.A.
09-01840		Germany	N.A.	01.06.2009	t034	V	Pig		PEN, OXA, ERY, TET, CIP i, OXaSu	N.A.
09-02165†	1	Germany	N.A.	01.06.2009	t034	V	Pig		PEN; OXA; CLI; TET	F
09-02423†		Germany	N.A.	01.06.2009	t011	III	Human	Commensal	PEN; OXA; ERY; CLI; TET	N.A.
09-02427†	1	Netherlands	N.A.	01.06.2009	t034	MSSA	Dog	Commensal	PEN; TET	N.A.
09-02428†	2	Netherlands	F 1	01.06.2009	t011	IV	Dog	Commensal	PEN; OXA; GEN; ERY; CLI; TET	C
09-02429†	8	Netherlands	F 1	01.06.2009	t011	IV	Dog	Commensal	PEN; OXA; GEN; ERY; CLI; TET	C
09-02431†	4	Netherlands	N.A.	01.06.2009	t1197	V	Horse	Commensal	PEN; OXA; ERY; CLI; TET; CIP; MFL	N.A.
09-02432†	5	Netherlands	F 1	01.06.2009	t011	IV	Horse	Commensal	PEN; OXA; GEN; ERY; CLI; TET; CIP; MFL	C
09-02434†	16	Netherlands	N.A.	01.06.2009	t011	V	Horse	Commensal	PEN; OXA; TET	N.A.
09-02435†	3	Netherlands	N.A.	01.06.2009	t011	V	Pig	Commensal	PEN; OXA; TET	N.A.
09-02436†	7	Netherlands	F 1	01.06.2009	t011	IV	Pig	Commensal	PEN; OXA; GEN; ERY; CLI; TET	C
09-02437†	12	Netherlands	N.A.	01.06.2009	t1457	V	Pig	Commensal	PEN; OXA; TET	N.A.
09-02438†	27	Netherlands	N.A.	01.06.2009	t011	V	Pig	Commensal	PEN; OXA; TET	N.A.
09-02439†	28	Netherlands	N.A.	01.06.2009	t1457	V	Pig	Commensal	PEN; OXA; TET	N.A.
09-02440†	9	Netherlands	F 1	01.06.2009	t011	IV	Bovine	Commensal	PEN; OXA; GEN; ERY; CLI; TET	C
09-02441†	10	Netherlands	N.A.	01.06.2009	t571	V	Bovine	Commensal	PEN; OXA; ERY; CLI; TET	N.A.
09-02442†	11	Netherlands	F 1	01.06.2009	t011	IV	Bovine	Commensal	PEN; OXA; GEN; ERY; CLI; TET	C
09-02444†	13	Netherlands	N.A.	01.06.2009	t108	V	Goat	Commensal	PEN; OXA; TET	B
09-02476†	44	Italy	F 3	30.07.2007	t899	IV	Human	Abscess	PEN; OXA; TET; SXT	N.A.
09-02477†	45	Italy	F 3	01.08.2007	t108	V	Human	Commensal	PEN; OXA; ERY; CLI; TET; CIP; SXT; MFL	B
09-02478†	46	Italy	F 3	01.08.2007	t899	MSSA	Human	Commensal	PEN; TET	N.A.
09-02611†	7	Germany	N.A.	01.06.2009	t034	V	Human	Commensal	PEN; OXA; TET	F

09-02615†	15	Germany	N.A.	01.06.2009	t034	V	Pig	Commensal	PEN; OXA; TET	F
09-03220	62931	Denmark	N.A.	21.10.2008	t011	V	Human		PEN, OXA, ERY, CLI, TET, SXT i, OX-aSu	F
09-03221	40850	Denmark	N.A.	11.03.2004	t034	Iva	Human		PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
09-03222	47772	Denmark	N.A.	16.12.2005	t034	IV	Human		PEN, OXA, TET, OXaSu	N.A.
09-03223	40315	Denmark	N.A.	26.01.2004	t034	V	Human		PEN, OXA, ERY, CLI, TET, SXT i, OX-aSu	N.A.
09-03224	40555	Denmark	N.A.	10.02.2004	t034	V	Human		PEN, OXA, ERY, CLI, TET, CIP i, SXT i, OXaSu	N.A.
09-03225	43511	Denmark	N.A.	26.01.2004	t034	V	Human		PEN, OXA, TET, SXT i, OXaSu	N.A.
09-03226	62939	Denmark	N.A.	21.10.2008	t034	IV	Human		PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
09-03227	51225	Denmark	N.A.	28.04.2006	t108	V	Human		PEN, OXA, TET, OXaSu	B
09-03229	52615	Denmark	N.A.	17.09.2006	t011	MSSA	Human		CIP i	N.A.
09-03230	45068	Denmark	N.A.	08.04.2005	t034	MSSA	Human		PEN, TET, CIP i, OXaSu	N.A.
09-03231	55241	Denmark	N.A.	27.01.2007	t571	MSSA	Human		ERY, CIP i	N.A.
09-03232	66369	Denmark	N.A.	23.05.2009	t1451	MSSA	Human		ERY, CIP i	N.A.
09-03233	62942	Denmark	N.A.	21.10.2008	t011	V	Human		PEN, OXA, GEN, ERY, CLI, TET, OX-aSu	N.A.
09-03234	50148	Denmark	N.A.	18.01.2006	t034	V	Human		PEN, OXA, ERY, CIP, MFL, OXaSu	N.A.
09-03235	58476	Denmark	N.A.	18.10.2007	t108	V	Human		PEN, OXA, TET, OXaSu	B
09-03236	66801	Denmark	N.A.	03.07.2009	t571	V	Human		PEN, OXA, TET, OXaSu	N.A.
09-03237	53783	Thailand	N.A.	01.09.2007	t034	IX	Human		PEN, OXA, TET, SXT i, OXaSu	N.A.
09-03238	53790	Canada	N.A.	28.09.2007	t034	X	Human		PEN, OXA, GEN, TET, OXaSu	N.A.
09-03323†	VB963847	Germany	N.A.	28.09.2007	t1344	nt	Dog		PEN; OXA; TET	N.A.
09-03324†	VB972261	Austria	N.A.	28.09.2007	t011	IV	Horse	Infection	PEN; OXA; GEN; TET	C
09-03325-1†	VB 972715.3	Germany	N.A.	28.09.2007	t011	IV	Horse	Clinical isolate	PEN; OXA; GEN; TET; CIP; SXT; MFL	C
09-03326†	VB976023.2	Austria	N.A.	28.09.2007	t011	IV	Horse	Infection	PEN; OXA; GEN; TET; SXT	C
09-03327†	VB976086.1	Germany	N.A.	28.09.2007	t034	nt	Cat		PEN; OXA; ERY; CLI; TET	A
09-03329	73-11242-1	Denmark	N.A.	01.06.2001	t034	MSSA	Pig		ERY, CLI, TET	N.A.
09-03330	74-13727-1	Denmark	N.A.	01.06.2002	t034	MSSA	Pig		PEN, ERY, CLI, TET	N.A.

09-03331	74-13714-1	Denmark	N.A.	01.06.2002	t034	MSSA	Pig		PEN, TET, SXT	F
09-03332	75-12166-1	Denmark	N.A.	01.06.2003	t034	MSSA	Pig		PEN, ERY, CLI, TET, SXT i	N.A.
09-03333	76-11472-1	Denmark	N.A.	01.06.2004	t034	MSSA	Pig		PEN, OXA, TET, CIP, SXT, MFL, OX-aSu	N.A.
09-03334	76-11995-1	Denmark	N.A.	01.06.2004	t034	MSSA	Pig		PEN, ERY, CLI, TET, SXT i	N.A.
09-03335	77-11730-1	Denmark	N.A.	01.06.2005	t034	MSSA	Pig		PEN, SXT	N.A.
09-03336	2007-70-95-9	Denmark	N.A.	01.06.2007	t2876	MSSA	Pig		PEN, ERY, CLI, TET, SXT i	N.A.
09-03337	USA42	USA	N.A.	01.06.1993	t034	MSSA	Bovine	Mastitis	PEN, ERY, CLI, TET	N.A.
09-03339	21441-4S	Denmark	N.A.	01.06.2007	t034	V	Pig		PEN, OXA, ERY, CLI, TET, SXT i, OX-aSu	N.A.
09-03340	23221-1B2	Denmark	N.A.	01.06.2007	t034	V	Pig		PEN, OXA, TET, SXT, OXaSu	N.A.
09-03343	98699-M-23-B-1	Denmark	N.A.	01.06.2007	t034	MSSA	Pig		PEN, CLI, TET, SXT	N.A.
09-03345	2007-70-52-4	Denmark	N.A.	01.06.2007	t034	V	Pig		PEN, OXA, TET, SXT, OXaSu	N.A.
09-03347	10(72398)	Denmark	N.A.	01.06.2007	t034	V	Pig		PEN, OXA, ERY, CLI, TET, CIP, SXT, MFL, OXaSu	F
10-01698		Germany	S.C. 6	22.07.2010	t011	V	Horse	Clinical isolate	PEN, OXA, GEN, ERY, CLI, TET, OX-aSu	C
10-02048		Germany	N.A.	22.07.2010	t034	MSSA	Human	Furuncle	PEN, ERY, CLI, TET	N.A.
10-02213-1		Germany	S.C. 6	20.09.2010	t011	V	Horse	Wound infection	PEN, OXA, GEN, ERY, CLI, TET, OX-aSu	C
10-02592		Germany	N.A.	26.10.2010	t011	V	Goose		PEN, OXA, TET, CIP, OXaSu	N.A.
10-02593		Germany	N.A.	26.10.2010	t011	V	Goose		PEN, OXA, TET, CIP, OXaSu	N.A.
10-02655		Germany	N.A.	09.11.2010	t034	V	Chicken (thaw water)		PEN, OXA, ERY, CLI, TET, OXaSu	E
10-02658		Germany	N.A.	09.11.2010	t2576	V	Chicken (thaw water)		PEN, OXA, GEN, ERY, CLI, TET, SXT i, OXaSu	N.A.
10-02693	1025102	Germany	A.C. 12	05.11.2010	t034	V	Horse	Clinical isolate	PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
11-00014		Germany	N.A.	14.12.2010	t011	V	Bovine	Mastitis	PEN, OXA, GEN, TET, OXaSu	N.A.

11-00078		Germany	N.A.	14.12.2010	t034	MSSA	Human	Abscess	PEN, ERY, CLI	N.A.
11-00080		Germany	N.A.	14.12.2010	t571	MSSA	Bovine	Mastitis	Sens.	N.A.
11-00501		Germany	N.A.	10.02.2011	t034	V	Chicken (thaw water)		PEN, OXA, ERY, CLI, TET, OXaSu	E
11-00530		Germany	N.A.	14.02.2011	t034	V	Chicken (thaw water)		PEN, OXA, ERY, CLI, TET, OXaSu	E
11-00569		Germany	N.A.	17.02.2011	t011	V	Chicken (thaw water)		PEN, OXA, GEN, ERY, CLI, TET, OXaSu	D
11-00571		Germany	N.A.	17.02.2011	t011	V	Chicken (thaw water)		PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
11-00830	1028487	Belgium	S.C. 2	14.12.2010	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, ERY, CLI, TET, RAM, OXaSu	C
11-00833	1101194	Germany	A.C. 13	12.01.2011	t6867	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01113-1	1102964	Germany	A.C. 13	28.01.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01119	1108771	Germany	A.C. 2	21.03.2011	t034	V	Horse	Clinical isolate	PEN, OXA, ERY, CLI, TET, OXaSu	E
11-01120	1108947	Germany	A.C. 12	21.03.2011	t034	V	Horse	Clinical isolate	PEN, OXA, GEN, ERY, CLI, TET, OXaSu	N.A.
11-01123	1109769	Germany	S.C. 5	28.03.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OXaSu	C
11-01124	1109802	Germany	A.C. 12	29.03.2011	t034	V	Horse	Clinical isolate	PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
11-01125	1109932	Germany	S.C. 5	31.03.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OXaSu	C
11-01188	1110544	Germany	A.C. 3	05.04.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01189	1110582	Germany	S.C. 5	04.04.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OXaSu	C
11-01190	1110692	Germany	A.C. 1	05.04.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01191	1110739	Germany	A.C. 4	06.04.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-	C

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11-01192	1110845	Germany	A.C. 5	07.04.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-01550	1114446	Germany	A.C. 1	10.05.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01553	1112803	Germany	S.C. 3	26.04.2011	t034	V	Horse	Clinical isolate	PEN, OXA, ERY, CLI, TET, OXaSu	E
11-01925	1114648	Germany	A.C. 1	13.05.2011	t1451	V	Horse	Clinical isolate	PEN, OXA, TET, OXaSu	N.A.
11-01929	1115092	Germany	A.C. 1	17.05.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01931	1115795	Germany	A.C. 6	27.05.2011	t011	V	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01932	1115872	Germany	S.C. 5	26.05.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-01937	1116588	Germany	A.C. 1	04.06.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-01940	1117093	Germany	A.C. 14	10.06.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02211		Germany	N.A.	07.07.2011	t034	MSSA	Human	Abscess	PEN, ERY, CLI	N.A.
11-02277	1117232	Germany	A.C. 1	14.06.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02281	1118615	Germany	S.C. 4	30.06.2011	t779	II	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-02283	1118823	Germany	A.C. 13	01.07.2011	t6867	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02285	1119501	Germany	A.C. 7	11.07.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02287	1119504	Germany	A.C. 8	11.07.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02558	1119783	Germany	A.C. 9	16.07.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02560	1119963	Germany	A.C. 10	18.07.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, SXT i, OXaSu	C
11-02561	1119983	Germany	A.C. 6	19.07.2011	t011	V	Horse	Clinical isolate	PEN, OXA, GEN, TET, OXaSu	C
11-02564	1120152	Germany	A.C. 12	23.07.2011	t034	V	Horse	Clinical isolate	PEN, OXA, ERY, CLI, TET, OXaSu	N.A.
11-02801	1121483	Germany	S.C. 5	14.08.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-02802	1121495	Germany	A.C. 1	14.08.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-02804	1121988	Germany	S.C. 5	19.08.2011	t011	IV	Horse	Clinical isolate	PEN, OXA, GEN, TET, CIP, MFL, OX-aSu	C
11-02806	1122115	Germany	A.C. 11	23.08.2011	t011	V	Horse	Clinical isolate	PEN, OXA, GEN, ERY, CLI, TET, SXT i, OXaSu	N.A.

*Resistance against the following antibiotics was tested: PEN, penicillin G; OXA, oxacillin; TPL, teicoplanin; VAN, vancomycin; GEN, gentamicin; TET, tetracycline; CIP, ciprofloxacin; MFL, moxifloxacin; SXT, trimethoprim/sulfamethoxazole (cotrimoxazole; SXTi, intermediate resistance to SXT); ERY, erythromycin; CLI, clindamycin; RAM, rifampicin; DAP, daptomycin; MUP, mupirocin; LNZ, linezolid and TGC, tigecycline. **The veterinary care or facilities in this study were divided into stationary care (S.C., where the animals must be hospitalized for at least one night in order to receive medical treatment) or ambulatory care (A.C., medical care is provided to animals without being admitted to a hospital for treatment). Numbers in (Farm/Stationary or Ambulatory Care) represent the various Farms (F), Stationary, or Ambulatory Care. N.A. in Fedral state = Not Available; in Clade = Not Assigned; and in Farm/Stationary or Ambulatory Care = No information Available. †All the 112 CC398 isolates that were analysed by Anne Wittenberg using the same method (dHPLC).

Table A.2. Primers used for Multilocus Sequence Typing (MLST).

Housekeeping loci	Primer	Sequence 5´-3
Carbamate kinase (*arcC*)	*arcC-F*	TTGATTCACCAGCGCGTATTGTC
	arcC-R	AGGTATCTGCTTCAATCAGCG
Shikimate dehydrogenase (*aroE*)	*aroE-F*	ATCGGAAATCCTATTTCACATTC
	aroE-R	GGTGTTGTATTAATAACGATATC
Glycerol kinase (*glpF*)	*glpF-F*	CTAGGAACTGCAATCTTAATCC
	glpF-R	TGGTAAAATCGCATGTCCAATTC
Guanylate kinase (*gmk*)	*gmk-F*	ATCGTTTTATCGGGACCATC
	gmk-R	TCATTAACTACAACGTAATCGTA
Phosphate acetyltransferase (*pta*)	*pta-F*	GTTAAAATCGTATTACCTGAAGG
	pta-R	GACCCTTTTGTTGAAAAGCTTAA
Triosephosphate isomerase (*tpi*)	*tpi-F*	TCGTTCATTCTGAACGTCGTGAA
	tpi-R	TTTGCACCTTCTAACAATTGTAC
Acetyl coenzyme A acetyltransferase (*yqiL*)	*yqiL-F*	CAGCATACAGGACACCTATTGGC
	yqiL-R	CGTTGAGGAATCGATACTGGAAC

Table A.3. Primers used for Staphylococcal Cassette Chromosome *mec* (SCC*mec*) typing.

Primer	Specific for	Amplified stretches	Sequence (5′-3′)	Size (bp)
α2, 2	SCC*mec* I	*ccr*A1/β2	CCTATATCATCAATCAGTACGT	690
β2, 2-1		*ccr*A1/β2	GCCTTGATAATAGCCTTC	
α3, 2	SCC*mec* II, IV	*ccr*A2/β	AGGCATCAATGCACAAACACT	920
β2, 2-1		*ccr*A2/β2	CGAATGAAGTGAAAGAAAGTGG	
MECI P2	SCC*mec* II	MECI P2	ATCAAGACTTGCATTCAGGC	209
MECI P3		MECI P3	GCGGTTTCAATTCACTTGTC	
DCS F2	SCC*mec* III	DCS F2	CATCCTATGATAGCTTGGTC	342
DCS R1		DCS R1	CTAAATCATAGCCATGACCG	
RIF4 F3	SCC*mec* V	RIF4 F3	GTGATTGTTCGAGATATGTGG	243
RIF4 R9		RIF4 R9	CGCTTTATCTGTATCTATCGC	
RIF5 F10		RIF5 F10	TTCTTAAGTACACGCTGAATCG	414
RIF5 R13		RIF5 R13	GTCACAGTAATTCCATCAATGC	
IS431 P4		IS431 P4	CAGGTCTCTTCAGATCTACG	381
pUB110 R1		pUB110 R1	GAGCCATAAACACCAATAGCC	
IS431 P4		IS431 P4	CAGGTCTCTTCAGATCTACG	303
pT181 R1		pT181 R1	GAAGAATGGGGAAAGCTTCAC	
MECA P4		MECA P4	TCCAGATTACAACTTCACCAGG	162
MECA P7		MECA P7	CCACTTCATATCTTGTAACG	

Table A.4. Primers used for screening of Mobile genetic elements (MGEs).

MGE	Primer	Sequence 5´-3	Reference
φAvβ1	*φAvβ-F1*	GCTTTGACATTTTCGGCATT	Lowder v, 2009
	φAvβ-R1	CTGGCTTGCTTTCTTCTGCT	
φAvβ2	*φAvβ-F2*	TTAACCCACTCCGCAAATTC	Lowder v, 2009
	φAvβ-R2	ATCGCAAGAGACCAAGCAGT	
φSa2	*intSa2-F*	TCAAGTAACCCGTCAACTCGGAGA	Schijffelen J M, 2010
	intSa2-R	TGAAA´CCCTCTGTCAACATAGCTCGAA	
φSa3	*intSa3-F*	ATGAAGCTCTAGCTAAT	Price et al, 2012
	IntSa3-R	CACAACGCTCCCAATGTT	
φSa6	*intSa6-F*	GCCATCAATTCAAGGATAG	Schijffelen J M, 2010
	IntSa6-R	TCTGCAGCTGAGGACAAT	
PVL	*PVL-F*	ATCATTAGGTAAAATGTCTGGACATGATCCA	Lina G, 1999
	PVL-R	GCATCAAGTGTATTGGATAGCAAAAGC	

Table A.5. Genetic loci and PCR primers for dHPLC.

Locus	ORF	Annotation	Left Primer (5´-3´)	Right Primer (5´-3´)	Position in (S0385 Genome)		Product size (bp)	Temperature (°C)
au200	SA0016	adenylosuccinate synthase	TTCGTTTCGAAGGTAATTGG	CGTGCATTTTCAGGTAATTCTT	23216	23628	371	56.3
au201	SA0098	hypothetical protein, similar to aminoacylase	TGAAGATGAAACGGCGAAAT	CCTGTTTTCATTGTGCTCAT	110529	110948	380	57.3
au202	SA0113	hypothetical protein, similar to ornithine cyclodeaminase (EC 4.3.1.12)	AAACGCGTGTTTGTTTACGA	TTTTGTTGTTGTGCCTGTTGA	130244	130731	447	56.3
au204	SA0171	NAD-dependent formate dehydrogenase	TGCGCATGAATTACAACACA	TCTTCAATGCGTTTTTGTGC	194555	194982	428	55.4
au205	SA0182	hypothetical protein, similar to indole-3-pyruvate decarboxylas	TTAGTTGCAATGAGCCATCA	TTCAGCAACAGCAGGATTTT	213236	213690	415	54.3
au206	SA0202	hypothetical protein, similar to gamma-glutamyltranspeptidase precursor	TCTTGCGGCTAAAATTGGTAA	TGGCGCGAATATTTTTCAGT	239439	239855	376	56.3
au207	SA0220	hypothetical protein, similar to glycerophosphodiester phosphodiesterase	TGAAAAGCGCATTGTTCTTC	TCCATTTTGACGCATTTTCA	263008	263471	424	54.6
au208	SA0256	6-phospho-beta-glucosidase	CCGCAAATCAATTTGAAGGT	GCGCAAAATGCACAAAATAA	310060	310488	389	55.6
au209	SA0299	hypothetical protein, similar to carbohydrate kinase, PfkB family	TTGCAGAAACATCAAATCCTG	TCATTTTTGGGGAAGAAACC	355123	355563	400	56.0
au210	SA0311	hypothetical protein, similar to trimethylamine dehydrogenase (EC 1.5.99.7)	TCGAAATTTCCACACCATCA	CCGTAAACGCATCAACAAAA	369364	369789	386	57.7
au212	SA0346	hypothetical protein, similar to cystathionine beta-lyase	TGACAGCACCAGCAATTAAA	ATTCGCTTTTGCTTTTGCAT	405743	406170	388	56.2
au213	SA0376	GMP synthase	AAGTTGAACGTGCCAATGAA	TTGATCCCCAATTGCTTTATG	434800	435239	399	57.2
au214	SA0419	cystathionine gamma-synthase	CAAACATGGCTTTGCGTTTA	ATGCCACCTGTTGAATTTGA	480802	481293	452	56.5
au215	SA0431	NADH-glutamate synthase small subunit	AAGCGGGCATTACTTTCATT	AACGCATTGTTTGAACACCA	495821	496294	434	55.8
au218	SA0507	hypothetical protein, similar to N-acyl-L-amino acid amidohydrolase	TCAAATTTGGGATTATGGTGTG	CAAACCTCATGAAACCATTGA	592338	592835	455	54.9
au219	SA0512	branched-chain amino acid aminotroansferase homologue	AAATTTCACCTGCTGCACAA	TGCAACTTTTGCAAAGCCTA	599411	599841	391	56.6
au220	SA0537	hypothetical protein, similar to phosphomethylpyrimidine kinase, thiD homologue	AAGCTTTGCCACCTTTAATGA	TGCAAGCAGATTTGAAAACG	631634	632116	442	56.3
au222	SA0697	hypothetical protein, similar to glycerate kinase	CAGTTGCAAGCCAAATTGAA	TCGACTTGAAAAATCCGACA	795048	795547	460	55.6
au223	SA0730	2, 3-diphosphoglycerate- independentphosphoglycerate mutase	CGGAAATTTTTGCGAACAGA	TTCGATTGCTTTGATTGTCG	836703	837148	406	55.3
au224	SA0791	hypothetical protein, similar to glycerate dehydrogenase	TGACGTTGTTATGTGGCAAAA	TTAAACCCTTGCAACCTTCTT	896974	897414	399	56.8
au225	SA0859	thimet oligopeptidase homologue	TACTGAATTGCGCAAAGAATTG	ATGCAACTTCAGCGACAAAA	974341	974741	359	55.4
au226	SA0945	dihydrolipoamide S-acetyltransferase component of pyruvate dehydrogenase complex E2	AAAGAAATCGCAGCTGAACAA	TTTTGAGCAATACGGCCAAT	1073758	1074161	363	56.2
au227	SA1044	dihydroorotase	ATGAAGGGAAACGCAGTAAA	CAATTCCGAATGGTGCTTTT	1181330	1181731	362	56.4
au228	SA1115	riboflavin kinase / FAD synthase ribC	CAATGGCATTCGGATTTTTC	CATTCGCTTTTTGCAACTCA	1264780	1265262	443	53.8
au229	SA1177	transketolase	TTGGTTCACCGAATAAAGCA	TGGATTTGTTTGAACCAGCA	1339372	1339785	374	54.8
au230	SA1226	asd, aspartate semialdehyde dehydrogenase	AAAAAGCTGGTGCAATCGTT	TCGTTTCTTTGTCAAGCGTTA	1401265	1401747	442	56.1
au231	SA1308	30S ribosomal protein S1	ATGCACCAAAGTTTGCCAAT	TTCCGGCTTCACTAATTTCAA	1513993	1514478	445	54.8
au232	SA1412	oxygen-independent coproporphyrinogen oxidase III	TTCATGGCCATCTAATGCAA	CATTTGAAGCAAATCCTGATGA	1617553	1618016	422	54.6

au233	SA1496	glutamyl-tRNA reductase	ATTTCCCCTGCACCAATAAT	CGAATTGCCCATGAAGATTT	1704604	1705096	453	56.1
au234	SA1545	D-3-phosphoglycerate dehydrogenase	TGCAGTGAATGCACCTAAAA	TAACAATGCGCCAGTTTTTC	1766721	1767216	456	54.2
au236	SA1814	hypothetical protein, similar to succinyl-diaminopimelate desuccinylase	ATGTTGCACCCATGTTCAAA	CATCGATTTGATGTGCCATT	2053853	2054311	419	55.6
au237	SA1924	hypothetical protein, similar to aldehyde dehydrogenase	GCAAATGCTGCTGCTAATTTT	TTAATGGCGAATGGGTAGAAA	2173216	2173675	418	56.7
au238	SA1991	6-phospho-beta-galactosidase	TTTTGTTGGCAATGCGTGTA	ATTTCAATTGCATGGTCACG	2263407	2263853	407	55.1
au239	SA2120	hypothetical protein, similar to amino acid amidohydrolase	GCAATTGCTTTATGCGTCAAT	TCAAAACAGGGGCAATTACA	2383969	2384377	368	57.3
au240	SA2214	adenosylmethionine-8-amino-7-oxononanoate aminotransferase	ACCCTGCGATTTCATCATTT	TAAAATTGCCCATTCTACGC	2485384	2485811	388	55.7
au242	SA2410	nrdD, anaerobic ribonucleoside-triphosphate reductase	ACTCGTGCGCAAATTTCTTT	ACGGATCGTTTTTGTCGTTT	2701426	2701886	421	56.5
au243	SA2470	hypothetical protein, similar to histidinol dehydrogenase	TGTTTGAATCGACGCATGTT	TTGCTGCATTGACTTATGGAA	2781905	2782326	381	57.2
au244	SA0177	argJ, arginine biosynthesis bifunctional protein homologue	TGGCAAATCACCAAGTTGAA	TGCGTTAATACGCACATAATCA	206898	207388	449	57.0
au245	SA0240	sorbitol dehydrogenase homologue	TTGAAAGTGCGAATGACGTT	CAAATATTCGTGCCCATTGA	291411	291896	446	57.9
au247	SA0361	truncated hypothetical protein, similar to phosphoglycerate mutase; Gpm3p	CATGGCGAATCAAAATCGAA	CCATCAAACAACGAATGACAA	418661	419144	443	55.2
au248	SA0527	probable glucosamine-6-phosphate isomerase	AAGAGATGGCATTTAATCAGCA	AATGGCTTGCTTTGGAACAT	622181	622659	437	54.6
au250	SA0572	hypothetical protein, similar to esterase/lipase	TTTAAACGATCACGTTGAGGA	AAAGGATCATGTTCCCCATT	665507	665947	400	54.2
au251	SA0671	hypothetical protein, similar to urea amidolyase	GAAATTGTGCCATCAGAATCA	TTTAAGCGTGCGAAATCTGA	764197	764696	459	54.2
au252	SA0821	argH, argininosuccinate lyase	TGCATTTTCAACTGGCTCAT	TTGACGTCGTTTCAAGCAA	926525	925992	494	54,8
au253	SA0896	menD, menaquinone biosynthesis protein	GGTTAGAAAAAATGGCAATGCT	GCCAAACAACCGTTCAAAAT	1017529	1017968	399	54.1
au254	SA0924	purN, phosphoribosylglycinamide formyltransferase	CGATTTTTGCATCAGGTTCA	TTTTGAATCGTCCGGTCTAA	1049523	1050001	439	54.8
au255	SA1089	sucD, succinyl-CoA synthetase	AAAAATGAAACTGGGGCAAC	CCGATTTCACCAATCATAACAA	1232627	1233078	410	56.9
au256	SA1150	glnA, glutamine-ammonia ligase	TGAAAAAGGGGAACCAACTT	AATCCGCGTGCATTTTTAAG	1307593	1308024	392	56.1
au257	SA1366	glycine dehydrogenase subunit 1	TCGCATTGCAAGCAAGAATA	CAATTGAACCGTAAAAGTTTGG	1575017	1575507	449	55.6
au258	SA1487	folC, folylpolyglutamate synthase	TTTACGTCGCCGTTTATTGA	CAATTGGCTTTGCATGTTGT	1693267	1693720	414	55.8
au259	SA1524	malate dehydrogenase homologue	CGCAATTTTTGAAGGACGTT	CTTTAGCAACATTACGAGCAA	1739355	1739845	450	56.2
au260	SA1554	acsA, acetyl-CoA synthetase	ATTGTTGGGCCGTTATTTGA	TTTGTAGCGCCATTTAACCA	1781281	1781729	409	55.8
au261	SA1585	proline dehydrogenase homologue	TTTAGGGGAATTTGTCGGTA	GAAATTGCGTGCGTTTAACA	1824023	1824497	435	54.7
au262	SA1669	citG, fumarate hydratase, class-II	ACACATTTACAAGATGCAACG	TGCAACCATTGTTAACATTTCA	1905900	1906352	410	57.2
au263	SA1735	manganese-dependent inorganic pyrophosphatase	CCTGAAATTGCCGGTTTAAT	TTTTTCGAGAAACAACACCAGA	1987761	1988244	442	55.1
au264	SA1864	3-isopropylmalate dehydratase large subunit	TGCATTGGAATTGGAACAA	TGATTTCAGGGAATGGTTCA	2105530	2105983	454	56,1
au265	SA1965	glmM, femD, phosphoglucosamine-mutase	ACAAAGCGCTTGAACAAGAA	TTGTGCAAATCTTTCAGCATC	2225913	2226346	393	55.4
au267	SA2071	moeB, molybdopterin biosynthesis protein moeB	TGCGTTGATTATTGGTATGG	TTTAATGCTGGCAATTGTGG	2332305	2332760	416	56.0
au268	SA2099	hypothetical protein, similar to monooxygenase	ATCCGGATTGTGCAAAAGAA	TGGGCAATTTTACCGATTTT	2359332	2359800	429	54.7
au269	SA2240	hypothetical protein, similar to para-nitrobenzyl esterase chain A	TGCACGCAATAAAGCACAAT	TGCAAATTCAGCAAGCCATA	2517061	2517511	411	55.0
au270	SA2260	hypothetical protein, similar to glucose 1-dehydrogenase	TGCGTGGGACAATTTTAATG	TTCCACGAATCATCACTTAAC	2537713	2538172	419	57.6
au271	SA2327	hypothetical protein, similar to pyruvate oxidase	ATGTACGATGCCAAAATGGA	TCATCCGGCAAGATTGTTTT	2612925	2613400	436	54.9
au272	SA2348	crtN, squalene desaturase	TTAATGCCAGATTTTGCACCT	TTTCGCATGATACGTTTGCT	2638403	2638864	421	54.3
au273	SA2393	hypothetical protein, similar to 2-dehydropantoate 2-	AGACACGCCAAAACAATAACA	ATGCATGATTGCAACTGTCT	2680864	2681340	436	55.7

		reductase						
au274	SA2490	pckA, phosphoenolpyruvate carboxykinase	TTTCAACTTTCGACGACACAA	TGCTTTAAAATGTGGTGCAGA	1844849	1845280	390	54.4
au275	SA0969	hypothetical protein, similar to glycerophosphoryl diester phosphodiesterase	CGCACCACAAATTATGTTTGA	TTCGGGATTGTTTGTTTGAA	1099466	1099873	367	55.6
au276	SA1334	hypothetical protein, similar to pyrroline-5-carboxylate reductase	AACACGCATCAACCACATA	TTTTTCTTAATTGAGCCATGC	1540756	1541157	402	55,7
au277	SA2439	sasF, conserved hypothetical protein (Cooper + Feil)	AATGCCTCAACGCAACATTA	CAACGATTTGGCGATTTGAT	2743068	2743554	447	54.9
au279	SA0740	hypothetical protein (Cooper + Feil)	TTGGAACATTTATTATGGCTTT	ACGGTCGATTTGACCTTTTA	847062	847504	401	54.0
au280	SA0817	hypothetical protein, similar to NADH-dependent flavin oxidoreductase (Cooper + Feil)	TGAAGAAGTGCTTAAAGCGAAA	AATTTCATCTTCCCGTCCAT	921075	921477	361	57.0
au281	SA2445	hypothetical protein (Cooper + Feil)	TTAAAATGGCCGGATGATGT	TTTCGCTGGAATATGATTGG	2753158	2753616	419	55.4
au282	SA2121	hutI, imidazolonepropionase (cooper + Feil)	CCCATTTAACATTTGGTGGA	GCTTCTTTGGCTTTTTGCAT	2385678	2386152	435	56.0
au284	SA0015	dnaC, replicative DNA helicase (Cooper + Feil)	AAAGTTGCAACGCATGAAGA	TTGCTCAATCGAACCAGATT	21445	21939	455	56.8
au285	SA0119	hypothetical protein, similar to diaminopimelate	TTGAATGTGGGCGCTTTATT	TCCTCCTTTGTTTGCGTTAAA	138812	139214	362	56.3
au286	SA0223	hypothetical protein, similar to UDP-glucose 4-epimerase	TGCAATCGTTTGAGAAGCAA	TGGTCGGTTCAAAGATGAAA	267449	267872	384	57.7
au287	SA0416	hypothetical protein, similar to carboxylesterase	ATTAAAATTGGCGCAGCACT	GCATTGAAAAAGCGAATAACA	477346	477781	395	55.0
au288	SA0545	phosphotransacetylase	GCGCAAAAACTTGATCTTGA	TGCCAAAGCTTAATGCTGATT	638508	638919	371	56.4
au289	SA0656	probable N-acetylglucosamine-6-phosphate deacetylase	ATTGTTGCAATTGGAGAAGG	ACGTTTCAAGCGCTTCTTTT	750368	750863	456	54.9
au290	SA0843	3-oxoacyl- synthase	TATGGCAATTGCAGGTTTCA	TTGCTTCAATTCCACCTGTT	956696	957156	421	57.8
au291	SA0881	hypothetical protein, similar to nucleotidase	GGCATCAAAACGTTGGATTA	GCGAAAATGGATGTGTTTGA	1001452	1001936	445	54.6
au292	SA0902	HisC homologue	TGGAACGATTGCTAAATGTGTT	TCCTTCGCTGAATTTGAAGA	1023317	1023721	363	54.8
au293	SA0958	myo-inositol-1(or 4)-monophosphatase homologue	TTTATGGATTTGCCCAAGGA	TTCAATGGTTTCAAGGGTTG	1084827	1085247	381	54.8
au294	SA1121	hypothetical protein, similar to processing proteinase	AAGAAATGGCCAACCGATAA	TTGCCGACAACATAAACAGAA	1273973	1274433	420	53.2
au295	SA1163	aspartate kinase homologue	AACAAAAATACCCGCTTCCTT	AAAGTTTCCAAATTCGGTGGT	1324852	1325298	405	53.9
au296	SA1199	hypothetical protein, similar to anthranilate synthase	AAATCCAACCATTCATGCCTA	TTCGCTGCATTCTTTTGGAT	1373094	1373518	384	55.3
au298	SA1349	dihydrolipoamide dehydrogenase	TTGATCCAACATGTGCCAAT	TGGTGTTATCGGAATGGAAT	1559232	1559653	382	54.6
au299	SA1377	glucokinase	TCAAATTGAGCAGGTGCAAA	GCGCAGAAATAGGTCATTTT	1582274	1582676	363	54.7
au300	SA1652	uroporphyrinogen decarboxylase	AAGCAAACCATGTTGCTTCA	TTCGCAACCAGAATATCGAA	1886778	1887220	403	55.3
au301	SA1685	hypothetical protein, similar to A/G-specific adenine	AATTGCCACATACCATGCAA	TCCCAAAGATCCTGATCAATTT	1932656	1933123	426	54.6
au302	SA1724	adenylosuccinate lyase	ACGGAAAGGTGTTTTCGTTT	AAATTTGCCGTTGAAATTCG	1974092	1974559	428	55.6
au303	SA1749	hypothetical protein, similar to aspartate transaminase	GCCCAAAGCTTAAATGAACAA	ATGTTTGAAGGTTTGCACCA	2001475	2001949	434	55.4
au304	SA1858	dihydroxy-acid dehydratase	TTGGTTTGACGGCGTATTTT	ATGCATCATCAATTGCTTCG	2097921	2098372	412	57.5
au305	SA1874	alanine racemase	TCGCATTGATGACCATTTACA	CTTGTATTTGAAGGCGTGTTT	2122020	2122457	396	55.8
au306	SA1963	mannitol-1-phosphate 5-dehydrogenase	TTGCAGACGTCAATGAAGAAA	TCTTTTTCAACAACCCATTCG	2216805	2217249	403	55.2
au307	SA2001	hypothetical protein, similar to oxidoreductase	TTCTTCTTGTTGATGGAATGGA	AAACAGCAGAAGCGGTTAAA	2272199	2272623	383	55.1
au308	SA2140	hypothetical protein, similar to esterase	TGCCATTAATTGGCGATATG	TTCCATTGGAGATTGTTGGA	2404832	2405246	375	55.8
au309	SA2188	nitrite reductase	TGCATTTCCATCAACCATTC	TAACCGCATCATGCTTTCAA	2459852	2460274	383	55.7

Table A.6. CC398 isolates used in the whole genome sequencing approach.

Isolate	Country	Isolation year	Host	Case	*spa*	SCC*mec*	tet(M)	Reference
06-02016	Austria	2006	Horse	Infection	t011	IVa	Positive	RKI, this study
08-00301	Germany	2007	Pig	Colonisation	t011	V	Positive	RKI, this study
10-02693	Germany	2010	Horse	Infection	t034	V	Positive	RKI, this study
11-00833	Germany	2011	Horse	Infection	t6867	IVa	Positive	RKI, this study
11-01925	Germany	2011	Horse	Infection	t1451	V	Positive	RKI, this study
11-02806	Germany	2011	Horse	Infection	t011	V	Positive	RKI, this study
IMT25053	Germany	2011	Horse	Infection	t011	V	Positive	FUB, this study
IMT26596	Germany	2011	Horse	Infection	t6867	IVa	Positive	FUB, this study
9B	Denmark	2007	Pig	Colonisation	t034	Vc (5C2&5)	Positive	Price et al, 2012
55488	Denmark	2007	Human	Colonisation	t034	Vc (5C2&5)	Positive	Price et al, 2012
PR7/08	Portugal	2007	Pig (dust)		t011	Vc (5C2&5)	Positive	Price et al, 2012
Veo8/003845st	Spain	2008	Pig (dust)		t011	Vc (5C2&5)	Positive	Price et al, 2012
UB08187	France	2008	Pig (dust)		t011	Vc (5C2&5)	Positive	Price et al, 2012
23824	Austria	2008	Pig (dust)		t011	Vc (5C2&5)	Positive	Price et al, 2012
AV6	Belgium	2008	Horse	Colonisation	t011	Vc (5C2&5)	Positive	Price et al, 2012
AV4	Belgium	2008	Horse	Colonisation	t1451	IVa (2B&5)	Positive	Price et al, 2012
29139	Italy	2008	Pig (dust)		t011	IVa (2B)	Positive	Price et al, 2012
M2009_10004208	Hungary	2009	Pig (dust)		t011	IVa (2B)	Positive	Price et al, 2012
ST398SO385	The Netherlands	2006	Human	Infection	t011	Vc (5C2&5)	Positive	Price et al, 2012
51225	Denmark	2006	Human	Colonisation	t108	Vc (5C2&5)	Positive	Price et al, 2012
F20	USA	2008	Pig (meat)		t034	MSSA	Positive	Price et al, 2012
P23-03_SW181.1	USA	2008	Pig	Colonisation	t034	Vc (5C2&5)	Positive	Price et al, 2012
F38	USA	2008	Pig	Colonisation	t034	MSSA	Positive	Price et al, 2012
F10	USA	2008	Pig (meat)		t034	MSSA	Positive	Price et al, 2012
P23-11_HF-446	China	2007	Human	Infection	t034	MSSA	Negative	Price et al, 2012
P23-9_WZ-1	China	2002	Human	Infection	t571	MSSA	Negative	Price et al, 2012
ST20091526	France	2009	Human	Infection	t571	MSSA	Negative	Price et al, 2012
LY19990171	France	1999	Human	Infection	t571	MSSA	Negative	Price et al, 2012

Table A.7. The investigated genetic loci and their detected polymorphisms.

Locus	Polymorphisms	Quality	Ancestral	Derived	Position in CC398 genome
au200	au200-1	Non- Synonymous	C	T	23542
	au200-2	Synonymous	A	G	23313
	au200-3	Synonymous	C	T	23466
au201	au201-1	Synonymous	A	G	116488
	au201-2	Synonymous	C	T	116566
	au201-3	Synonymous	T	A	116596
	au201-4	Non- Synonymous	C	T	116694
au202	au202-1	Non- Synonymous	G	A	133896
	au202-2	Synonymous	C	T	134025
	au202-3	Synonymous	T	C	134127
	au202-4	Synonymous	T	A	134217
	au202-5	Synonymous	A	G	134232
	au202-6	Non- Synonymous	C	A	133893
	au202-7	Non- Synonymous		Deletion 14 bp CACACGCTTCAGAG	133887-133900
	au203-1	Non- Synonymous	C	T	146630
au204	au204-1	Non- Synonymous	T	C	198844
au206	au206-1	Non- Synonymous	T	A	239258
au207	au207-1	Non- Synonymous	G	A	263000
	au207-2	Non- Synonymous	T	C	262976
au208	au208-1	Synonymous	G	A	313878
au209	au209-1	Non- Synonymous	C	T	358137
au210	au210-1	Non- Synonymous	C	T	419613
au212	au212-1	Non- Synonymous	T	A	456070
	au212-2	Non- Synonymous	G	A	456279
au214	au214-1	Non- Synonymous	A	T	539521
	au214-2	Non- Synonymous	C	A	539264
au215	au215-1	Synonymous	C	T	554340
	au215-2	Synonymous	A	T	508003
au223	au223-1	Non- Synonymous	A	G	901302
au227	au227-1	Non- Synonymous	C	T	1251377
au228	au228-1	Non- Synonymous	T	C	1333553
au230	au230-1	Non- Synonymous	T	G	1470360
au231	au231-1	Non- Synonymous	C	A	1583161
au232	au232-1	Non- Synonymous	T	G	1731536
au234	au234-1	Synonymous	G	A	1872909
	au234-2	Non- Synonymous	C	T	1872581
	au234-3	Non- Synonymous	A	T	1872820
au236	au236-1	Non- Synonymous	C	A	2137364
	au236-2	Synonymous	G	A	2137214
au237	au237-1	Synonymous	T	C	2241862
au239	au239-1	Synonymous	T	A	2455456
	au239-2	Non- Synonymous	G	A	2455634

au240	au240-1	Non- Synony-mous	G	A	2558218
au243	au243-1	Non- Synony-mous	G	C	2840526
	au243-2	Non- Synony-mous	C	T	2840670
au244	au244-1	Synonymous	G	A	207306
	au244-2	Non- Synony-mous	A	Deletion of one A	210973
au245	au245-1	Non- Synony-mous	G	A	292470
au247	au247-1	Non- Synony-mous	A	C	467424
au251	au251-1	Non- Synony-mous	T	A	827455
	au251-2	Non- Synony-mous	G	A	827793
au252	au252-1	Non- Synony-mous	T	A	982524
	au252-2	Synonymous	C	T	982720
	au252-3	Non- Synony-mous	G	A	982789
au254	au254-1	Non- Synony-mous	C	T	1122187
au255	au255-1	Synonymous	T	C	1302242
	au255-2	Synonymous	A	G	1302390
au256	au256-1	Non- Synony-mous	G	A	1377726
	au256-2	Synonymous	T	C	1377565
	au256-3	Synonymous	C	T	1377735
au257	au257-1	Non- Synony-mous	C	A	1688944
	au257-2	Non- Synony-mous	A	G	1688994
au260	au260-1	Synonymous	A	T	1887201
au261	au261-1	Synonymous	T	C	1929639
au262	au262-1	Non- Synony-mous	C	T	2012944
au264	au264-1	Synonymous	G	A	2174508
au265	au265-1	Synonymous	T	C	2299872
au267	au267-1	Non- Synony-mous	C	T	2403403
au269	au269-1	Non- Synony-mous	G	A	2589905
	au269-2	Non- Synony-mous		Insertion of one A	2589898
au270	au270-1	Non- Synony-mous	G	A	2609589
au273	au273-1	Synonymous	G	A	2735776
	au273-2	Non- Synony-mous	T	C	2735604
	au273-3	Non- Synony-mous	A	G	2735517
au279	au279-1	Non- Synony-mous	G	A	911481
	au279-2	Non- Synony-mous	T	C	911544
	au279-3	Synonymous	A	C	911498
au282	au282-1	Synonymous	G	A	2457315
au284	au284-1	Non- Synony-mous	G	T	21662
au286	au286-1	Non- Synony-mous	G	A	267557
au289	au289-1	Non- Synony-mous	T	G	813835
au291	au291-1	Non- Synony-mous	G	A	1074694
au294	au294-1	Non- Synony-mous	C	T	1342929
	au294-2	Non- Synony-mous	A	C	1342883
	au294-3	Non- Synony-mous	C	T	1342720

au296	au296-1	Non- Synony-mous		Insertion of 6 bp TATTTT	1441728-1441733
au298	au298-1	Synonymous	C	T	1673345
	au298-2	Non- Synony-mous	A	T	1673293
	au298-3	Synonymous	A	G	1673244
au300	au300-1	Synonymous	C	T	1993865
	au300-2	Non- Synony-mous	G	A	1993929
	au300-3	Non- Synony-mous	G	A	1994130
au304	au304-1	Non- Synony-mous	G	A	2166786
au307	au307-1	Synonymous	T	C	2346800
au309	au309-1	Non- Synony-mous	T	C	2533073
	au309-2	Synonymous	A	T	2533404

Table A.8. Additional human isolates screened for the SNP au309-2

Isolate ID	Country	Federal state	Hospital*	Isolation date	*Spa* type	Host	Characteristic	Nucleotide at position 2,533,404 (SNP au309-2)
10-00069	Germany	Hesse	1	28/12/09	t011	Human	Clinical isolate	A
10-00190	Germany	North Rhine-Westphalia	2	2010	t011	Human	Clinical isolate	A
10-00289	Germany	Bavaria	3	02/02/10	t011	Human	Clinical isolate	A
10-00307	Germany	Brandenburg	4	08/02/10	t011	Human	Clinical isolate	A
10-00345	Germany	Schleswig-Holstein	5	25/01/10	t011	Human	Clinical isolate	A
10-00825	Germany	Bavaria	3	05/04/10	t011	Human	Clinical isolate	A
10-00856	Germany	Lower Saxony	6	26/03/10	t011	Human	Clinical isolate	A
10-00901	Germany	Hesse	7	12/04/10	t011	Human	Clinical isolate	A
10-01083	Germany	Bavaria	8	04/05/10	t011	Human	Clinical isolate	A
10-01322	Germany	Lower Saxony	9	04/08/09	t011	Human	Clinical isolate	A
10-01346	Germany	Saxony	10	31/05/10	t011	Human	Clinical isolate	A
10-01433	Germany	Lower Saxony	11	2010	t011	Human	Clinical isolate	A
10-01458	Germany	Bavaria	12	21/06/10	t011	Human	Clinical isolate	T
10-01814	Germany	Saxony	10	20/07/10	t011	Human	Clinical isolate	A
10-01920	Germany	Schleswig-Holstein	13	02/08/10	t011	Human	Clinical isolate	A
10-01949	Germany	Bavaria	3	18/08/10	t011	Human	Clinical isolate	A
10-01972	Germany	North Rhine-Westphalia	14	03/08/10	t011	Human	Clinical isolate	A
10-02072-1	Germany	Bavaria	3	30/08/10	t011	Human	Clinical isolate	A
10-02423-1	Germany	Saxony	10	28/09/10	t011	Human	Clinical isolate	A
10-02477	Germany	Schleswig-Holstein	5	07/10/10	t011	Human	Clinical isolate	A
10-02565	Germany	Saxony-Anhalt	15	23/10/10	t011	Human	Clinical isolate	A
10-02573	Germany	Bavaria	3	22/10/10	t011	Human	Clinical isolate	A
10-02581	Germany	Brandenburg	16	15/10/10	t011	Human	Clinical isolate	A
10-02855	Germany	Schleswig-Holstein	17	25/11/10	t011	Human	Clinical isolate	A
11-00322	Germany	North Rhine-Westphalia	18	21/01/11	t011	Human	Clinical isolate	A
11-00496	Germany	Saxony-Anhalt	15	05/02/11	t011	Human	Clinical isolate	A
11-00666	Germany	Schleswig-Holstein	17	23/02/11	t011	Human	Clinical isolate	A
11-00708	Germany	Bavaria	3	28/02/11	t011	Human	Clinical isolate	A
11-00720	Germany	Bavaria	3	01/03/11	t011	Human	Clinical isolate	T
11-00776	Germany	Lower Saxony	11	25/02/11	t011	Human	Clinical isolate	A
11-00893	Germany	Schleswig-Holstein	17	17/03/11	t011	Human	Clinical isolate	A
11-00896	Germany	Lower Saxony	11	23/02/11	t011	Human	Clinical isolate	A
11-01155	Germany	Lower Saxony	19	03/04/11	t011	Human	Clinical isolate	A
11-01170	Germany	Hamburg	20	02/04/11	t011	Human	Clinical isolate	A
11-01298	Germany	Brandenburg	21	2011	t011	Human	Clinical isolate	A
11-01869	Germany	Berlin	22	2011	t011	Human	Clinical isolate	A
11-02050	Germany	Schleswig-Holstein	5	25/05/11	t011	Human	Clinical isolate	A
11-02153	Germany	Hesse	1	14/06/11	t011	Human	Clinical isolate	A
11-02310	Germany	North Rhine-Westphalia	2	28/03/11	t011	Human	Clinical isolate	A
11-02359	Germany	Mecklenburg-Western Pomerania	23	22/07/11	t011	Human	Clinical isolate	A
11-02636	Germany	Lower Saxony	19	18/08/11	t011	Human	Clinical isolate	T
11-02735	Germany	Schleswig-Holstein	24	26/08/11	t011	Human	Clinical isolate	T

11-03257	Germany	Schleswig-Holstein	25	04/11/11	t011	Human	Clinical isolate	A
11-03267	Germany	Baden-Württemberg	26	2011	t011	Human	Clinical isolate	A
11-03381	Germany	North Rhine-Westphalia	27	2011	t011	Human	Clinical isolate	A
11-03422	Germany	Schleswig-Holstein	28	11/11/11	t011	Human	Clinical isolate	A
11-03616	Germany	Hesse	1	07/12/11	t011	Human	Clinical isolate	A
11-01712	Germany	Hesse	29	19/05/11	t011	Human	Clinical isolate	A

*Numbers in Column E (Hospital) represent the various Hospitals that the isolates were collected from

Table A.9. The 195 investigated CC398 and their acquisition to various bacteriophages.

Isolate ID	Host	*Spa* type	SCC*mec*	φAvβ	φSa2	φSa3	φSa6	lukPV
04-03094	Human	t034	V	Negative	Negative	Negative	Positive	Negative
05-02057	Dog	t034	V	Negative	Negative	Negative	Positive	Negative
06-00262	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
06-00880	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
06-00903	Human	t011	V	Negative	Positive	Negative	Negative	Negative
06-02016	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
06-02985	Human	t011	V	Negative	Positive	Negative	Negative	Negative
06-03005	Human	t571	MSSA	Negative	Negative	Positive	Negative	Negative
06-03034	Human	t034	V	Negative	Negative	Negative	Positive	Negative
07-00334	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-00415	Human	t034	MSSA	Negative	Positive	Negative	Negative	Negative
07-00471	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-00755	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
07-00757	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01238	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01239	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01274	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
07-01335	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01337	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01388	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01429	Human	t571	MSSA	Negative	Negative	Positive	Negative	Negative
07-01494	Human	t034	V	Negative	Negative	Negative	Negative	Negative
07-01640	Human	t034	V	Negative	Negative	Negative	Positive	Negative
07-01653	Human	t034	V	Negative	Negative	Negative	Positive	Negative
07-01730	Human	t011	IV	Negative	Positive	Negative	Positive	Negative
07-01826	Human	t1197	MSSA	Negative	Negative	Negative	Negative	Negative
07-01949	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
07-02239	Human	t108	V	Negative	Positive	Negative	Negative	Negative
07-02347	Human	t034	nt	Negative	Negative	Negative	Positive	Negative
07-02415	Pig	t2974	V	Negative	Negative	Negative	Negative	Negative
07-02424	Pig	t011	V	Negative	Positive	Negative	Negative	Negative
07-02431	Human	t011	V	Negative	Positive	Negative	Negative	Negative
07-02432	Human	t011	V	Negative	Positive	Negative	Negative	Negative
07-02433	Human	t011	V	Negative	Negative	Negative	Negative	Negative
07-02464	Human	t034	MSSA	Negative	Negative	Negative	Positive	Negative
07-02558	Human	t034	V	Negative	Positive	Negative	Positive	Negative
07-02632	Human	t034	V	Negative	Negative	Negative	Positive	Negative
07-02642	Human	t034	V	Negative	Negative	Negative	Positive	Negative
07-03026	Human	t011	V	Negative	Positive	Negative	Negative	Negative
07-03443	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
08-00301	Pig	t011	V	Negative	Positive	Negative	Negative	Negative
08-00306	Human	t011	V	Negative	Positive	Negative	Negative	Negative
08-00307	Human	t011	V	Negative	Positive	Negative	Negative	Negative
08-00308	Human	t011	V	Negative	Positive	Negative	Negative	Negative
08-00360	Human	t034	MSSA	Negative	Positive	Negative	Negative	Negative
08-00401	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
08-00537	Turkey	t034	MSSA	Positive	Negative	Negative	Positive	Negative
08-00543	Turkey	t034	MSSA	Positive	Negative	Negative	Positive	Negative
08-00700	Human	t011	MSSA	Negative	Positive	Negative	Negative	Negative
08-00798	Turkey	t034	MSSA	Positive	Negative	Negative	Positive	Negative
08-00888	Turkey	t034	MSSA	Positive	Negative	Negative	Positive	Negative
08-00907	Human	t011	V	Negative	Positive	Negative	Negative	Negative
08-00912	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
08-01058	Human	t034	MSSA	Negative	Negative	Negative	Positive	Negative
08-01223	Human	t034	MSSA	Negative	Positive	Negative	Positive	Negative
08-01388	Human	t011	V	Negative	Positive	Negative	Negative	Negative
08-01605	Human	t011	MSSA	Negative	Negative	Negative	Positive	Negative
08-01712	Pig	t011	V	Negative	Positive	Negative	Negative	Negative
08-01737	Pig	t011	V	Negative	Negative	Negative	Negative	Negative
08-01849	Human	t011	V	Negative	Negative	Negative	Negative	Negative
08-02208	Human	t034	V	Negative	Negative	Negative	Positive	Negative
08-02418	Human	t034	V	Negative	Negative	Negative	Positive	Negative
08-02710	Human	t034	MSSA	Negative	Negative	Negative	Positive	Negative
08-02807	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-00339	Human	t011	V	Negative	Positive	Negative	Negative	Negative
09-00340-1	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
09-00341	Pig	t011	V	Negative	Positive	Negative	Negative	Negative
09-00342	Chicken (thaw water)	t011	V	Negative	Positive	Negative	Negative	Negative
09-00343	Horse	t011	V	Negative	Positive	Negative	Negative	Negative

09-00393	Human	t034	V	Negative	Negative	Negative	Negative	Negative
09-00443	Human	t011	V	Negative	Positive	Negative	Negative	Negative
09-00444	Human	t011	V	Negative	Positive	Negative	Negative	Negative
09-00445	Human	t011	V	Negative	Positive	Negative	Negative	Negative
09-00709	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-00711	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-01308	Pig	t034	V	Negative	Positive	Negative	Negative	Negative
09-01309	Pig	t034	V	Negative	Positive	Negative	Negative	Negative
09-01310	Pig	t034	V	Negative	Positive	Negative	Positive	Negative
09-01311	Human	t034	MSSA	Negative	Negative	Positive	Negative	Negative
09-01312	Human	t571	MSSA	Negative	Negative	Positive	Negative	Negative
09-01313	Environment	t034	V	Negative	Negative	Negative	Negative	Negative
09-01314	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-01315	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-01316	Human	t034	V	Negative	Positive	Negative	Positive	Negative
09-01318	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-01840	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-02165	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-02423	Human	t011	III	Negative	Positive	Negative	Negative	Negative
09-02427	Dog	t034	MSSA	Negative	Negative	Negative	Negative	Negative
09-02428	Dog	t011	IV	Negative	Negative	Negative	Positive	Negative
09-02429	Dog	t011	IV	Negative	Negative	Negative	Positive	Negative
09-02431	Horse	t1197	V	Negative	Negative	Negative	Negative	Negative
09-02432	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
09-02434	Horse	t011	V	Negative	Positive	Negative	Negative	Negative
09-02435	Pig	t011	V	Negative	Negative	Negative	Positive	Negative
09-02436	Pig	t011	IV	Negative	Negative	Negative	Negative	Negative
09-02437	Pig	t1457	V	Negative	Positive	Negative	Negative	Negative
09-02438	Pig	t011	V	Negative	Positive	Negative	Negative	Negative
09-02439	Pig	t1457	V	Negative	Positive	Negative	Negative	Negative
09-02440	Bovine	t011	IV	Negative	Positive	Negative	Negative	Negative
09-02441	Bovine	t571	V	Negative	Positive	Negative	Positive	Negative
09-02442	Bovine	t011	IV	Negative	Negative	Negative	Positive	Negative
09-02444	Goat	t108	V	Negative	Positive	Negative	Negative	Negative
09-02476	Human	t899	IV	Negative	Positive	Negative	Negative	Negative
09-02477	Human	t108	V	Negative	Positive	Negative	Negative	Negative
09-02478	Human	t899	MSSA	Negative	Positive	Negative	Negative	Negative
09-02611	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-02615	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-03220	Human	t011	V	Negative	Positive	Negative	Positive	Negative
09-03221	Human	t034	Iva	Negative	Negative	Negative	Positive	Negative
09-03222	Human	t034	IV	Negative	Negative	Negative	Positive	Negative
09-03223	Human	t034	V	Negative	Negative	Negative	Negative	Negative
09-03224	Human	t034	V	Negative	Positive	Negative	Positive	Negative
09-03225	Human	t034	V	Negative	Negative	Negative	Positive	Negative
09-03226	Human	t034	IV	Negative	Negative	Negative	Positive	Negative
09-03227	Human	t108	V	Negative	Negative	Negative	Negative	Negative
09-03229	Human	t011	MSSA	Negative	Positive	Positive	Negative	Negative
09-03230	Human	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03231	Human	t571	MSSA	Negative	Negative	Positive	Negative	Negative
09-03232	Human	t1451	MSSA	Negative	Negative	Negative	Negative	Negative
09-03233	Human	t011	V	Negative	Negative	Negative	Negative	Positive
09-03234	Human	t034	V	Negative	Positive	Positive	Positive	Positive
09-03235	Human	t108	V	Negative	Positive	Negative	Negative	Positive
09-03236	Human	t571	V	Negative	Positive	Negative	Positive	Positive
09-03237	Human	t034	IX	Negative	Negative	Negative	Positive	Negative
09-03238	Human	t034	X	Negative	Negative	Negative	Positive	Negative
09-03323	Dog	t1344	nt	Negative	Positive	Negative	Negative	Negative
09-03324	Horse	t011	IV	Negative	Positive	Negative	Negative	Negative
09-03325-1	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
09-03326	Horse	t011	IV	Negative	Negative	Negative	Negative	Negative
09-03327	Cat	t034	nt	Negative	Negative	Negative	Positive	Negative
09-03329	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03330	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03331	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03332	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03333	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03334	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03335	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03336	Pig	t2876	MSSA	Negative	Positive	Negative	Positive	Negative
09-03337	Bovine	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03339	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-03340	Pig	t034	V	Negative	Negative	Negative	Positive	Negative

09-03343	Pig	t034	MSSA	Negative	Negative	Negative	Positive	Negative
09-03345	Pig	t034	V	Negative	Negative	Negative	Positive	Negative
09-03347	Pig	t034	V	Negative	Positive	Negative	Positive	Negative
10-01698	Horse	t011	V	Negative	Negative	Negative	Negative	Negative
10-02048	Human	t034	MSSA	Negative	Positive	Positive	Negative	Positive
10-02213-1	Horse	t011	V	Negative	Positive	Negative	Negative	Negative
10-02592	Goose	t011	V	Negative	Positive	Negative	Negative	Negative
10-02593	Goose	t011	V	Negative	Positive	Negative	Negative	Negative
10-02655	Chicken (thaw water)	t034	V	Negative	Positive	Negative	Positive	Negative
10-02658	Chicken (thaw water)	t2576	V	Negative	Negative	Negative	Positive	Negative
10-02693	Horse	t034	V	Negative	Negative	Negative	Positive	Negative
11-00014	Bovine	t011	V	Negative	Positive	Negative	Negative	Negative
11-00078	Human	t034	MSSA	Negative	Positive	Positive	Negative	Positive
11-00080	Bovine	t571	MSSA	Negative	Positive	Negative	Negative	Negative
11-00501	Chicken (thaw water)	t034	V	Negative	Negative	Positive	Positive	Negative
11-00530	Chicken (thaw water)	t034	V	Negative	Negative	Positive	Positive	Negative
11-00569	Chicken (thaw water)	t011	V	Negative	Negative	Negative	Negative	Negative
11-00571	Chicken (thaw water)	t011	V	Negative	Positive	Negative	Negative	Negative
11-00830	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
11-00833	Horse	t6867	IV	Negative	Negative	Negative	Negative	Negative
11-01113-1	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01119	Horse	t034	V	Negative	Negative	Negative	Positive	Negative
11-01120	Horse	t034	V	Negative	Negative	Negative	Positive	Negative
11-01123	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01124	Horse	t034	V	Negative	Negative	Negative	Positive	Negative
11-01125	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
11-01188	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01189	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01190	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01191	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01192	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01550	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01553	Horse	t034	V	Negative	Negative	Positive	Positive	Negative
11-01925	Horse	t1451	V	Negative	Positive	Negative	Negative	Negative
11-01929	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01931	Horse	t011	V	Negative	Positive	Negative	Negative	Negative
11-01932	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01937	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-01940	Horse	t011	IV	Negative	Positive	Negative	Positive	Negative
11-02211	Human	t034	MSSA	Negative	Positive	Positive	Negative	Positive
11-02277	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-02281	Horse	t779	II	Negative	Negative	Positive	Positive	Negative
11-02283	Horse	t6867	IV	Negative	Negative	Negative	Negative	Negative
11-02285	Horse	t011	IV	Negative	Negative	Negative	Negative	Negative
11-02287	Horse	t011	IV	Negative	Negative	Negative	Negative	Negative
11-02558	Horse	t011	IV	Negative	Negative	Negative	Negative	Negative
11-02560	Horse	t011	IV	Negative	Negative	Negative	Negative	Negative
11-02561	Horse	t011	V	Negative	Positive	Negative	Negative	Negative
11-02564	Horse	t034	V	Negative	Negative	Negative	Positive	Negative
11-02801	Horse	t011	IV	Negative	Negative	Positive	Positive	Negative
11-02802	Horse	t011	IV	Negative	Negative	Positive	Positive	Negative
11-02804	Horse	t011	IV	Negative	Negative	Negative	Positive	Negative
11-02806	Horse	t011	V	Negative	Positive	Negative	Negative	Negative

LIST OF ABBREVIATIONS

Abbreviation	Description
AA	Amino acid
ATP	Adenosine triphosphate
APS	Adenosine 5' phosphosulfate
Bp, Kb, Mb	Base pair(s), Kilo-base pair(s), Mega-base pair(s)
BaTS	Bayesian tip-association significance testing
BLAST	Basic local alignment search tool
BRIG	Blast ring image generator
BURST	Based upon related sequence types
BWA	Burrows-wheeler alignment
°C	Celsius
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CC	Clonal complex
ccr	Cassette chromosome recombinase
CFSE	5 and 6- carboxyfluorescein diacetate succinimidyl ester
Chip	Chemotaxis inhibitory protein
Contigs	Contiguous sequences
ddNTPs	Dideoxynucleotide triphosphates
dHPLC	Denaturing high-performance liquid chromatography
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
ECM	Extracellular matrix
ELISA	Enzyme linked immuno-sorbent assay
ESS	Efective sample size
EUCAST	European committee on antimicrobial susceptibility testing
FACS	Flow cytometer
Fc	Fragment crystallisable
FN	Fibronectin
FnBP-A, FnBP-B	Fibronectin binding protein A, Fibronectin binding protein B
g, mg, µg, ng	Gram, milligram, microgram, nanogram
GS	Genome sequence
h, min, s	Hour, min, second
HA-MRSA	Healthcare-associated methicillin-resistant *Staphylococcus aureus*
HKY	Hasegawa-kishino-yano
IEC	Immune evasion cluste

IgG	Immunoglobulin G
Int	Integrase
J-regions	Joining regions
L, ml, µl	Litre, millilitre, microliter
LA-MRSA	Livestock-methicillin-resistant *Staphylococcus aureus*
LB	Luria bertani
MC	Maximum exclusive single-state clade size
MCMC	Markov chain monte carlo
MgCl2	Magnesium chloride
MGEs	Mobile genetic elements
MH	Mueller hinton
ML	Maximum likelihood
MLST	Multilocus sequence typing
MRSA	Methicillin-Resistant *Staphylococcus aureus*
MSSA	Methicillin- sensitive *Staphylococcus aureus*
NaCl, NaOH	Sodium chloride, Sodium hydroxide
NCBI	National Center for Biotechnology Information
ORFs	Open reading frames
PBP	Penicillin binding protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
PS	Parsimony score
PVL	Panton-valentine leukocidin
RKI	The Robert Koch institute
S. aureus	*Staphylococcus aureus*
Sak	Immune-modulating proteins staphylokinase
SaPI	*Staphylococcus aureus* pathogenicity island
SCC*mec*	Staphylococcal cassette chromosome *mec*
Scin	Staphylococcal complement inhibitor
SNP	Single-nucleotide polymorphism
Spa	Staphylococcal protein a
ST	Sequence type
TMRCA	Time to most recent common ancestor
TSB	Tryptic soy broth
UV	Ultraviolet
VNTR	Variable number of tandem repeats

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